INJURY COMPENSATION REVEALS IMPLICIT GOALS THAT GUIDE LOCOMOTOR COORDINATION

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INJURY COMPENSATION REVEALS IMPLICIT GOALS THAT GUIDE LOCOMOTOR COORDINATION

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To my parents, for always believing in me.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

θmtp	Metatarsophalangeal Joint Angle
$\theta ankle$	Ankle Joint Angle
$\theta knee$	Knee Joint Angle
θhip	Hip Joint Angle
$\theta limb$	Limb Angle
Llimb	Limb Length
PSIS	Posterior Superior Iliac Spine
R^2	Coefficient of Determination
CPG	Central Pattern Generator
PNI	Peripheral Nerve Injury

SUMMARY

Locomotion persists despite changes in external and internal circumstances. Motor responses to gait impairment exhibit commonalities across various taxa and types of injury, yet we lack a systematic understanding of compensation strategies. The objective of this dissertation is to uncover principles governing implicit goals within the control of locomotion. I propose that coordination of injured locomotion will demonstrate that these goals follow a hierarchical organization of the neuromuscular system. Accurate quantification of gait deficits in rodents demands sophisticated measurement techniques. I utilize X-ray technology to examine intralimb and interlimb coordination after unilateral injury in rats. My findings indicate that compensation to injury involves the coordination of lower-order motor elements to preserve the pre-injury behaviors of higher-order elements. Specifically I present evidence that preservation of limb angle and limb length are critical task goals that transcend injury states and afferent sensory feedback conditions. Broadening my investigation to include interlimb coordination revealed that task goals may change to satisfy the goals of a higher hierarchical level. This work is a necessary precursor to study locomotor coordination and injury compensation in more complex rodent injury models such as self-reinnervation, sciatic nerve, and spinal cord injury. These results could also translate to clinical gait rehabilitation through future protocols that address motor patterns of the entire limb over the behavior of individual joints.

CHAPTER 1

INTRODUCTION

Locomotion is robust. The capacity of an animal to traverse its environment persists through myriad changes in circumstance, both of external surroundings as well as internal physiological conditions. Despite profound advances in describing the mechanical and dynamic characteristics of locomotion, we lack a clear understanding of the underlying tasks that the neuromuscular system executes to produce movement. Coordination of the innumerable elements involved in locomotion likely makes use of hierarchical organizational principles to manage task goals at multiple anatomical levels. In this dissertation I investigate how changes in the coordination of post-injury gait yield insight into the implicit goals of motor control.

1.1 Representation of Locomotion

Descriptions of locomotion typically begin with quantifying an organism's general movement through space. The definitive measurement of locomotion is velocity, a vector relating the rate and direction of travel (Perry 1992). Walking velocity is an important clinical criterion in distinguishing between normal and pathological gait (Andriacchi et al. 1977). Spatiotemporal parameters, such as the length and duration of a single gait cycle or "stride," as well as the duration of the support or "stance" and non-weight bearing or "swing" phases of the cycle, correlate with walking velocity and also could act as detectors of gait impairment (Wagenaar and Beek 1992; Roth et al. 1997). These gross characterizations of locomotion are simple to collect in that only the contact between the endpoint of the limb and the ground must be measured, yet these techniques belie the potential variability in limb and joint motions. A more detailed picture of

locomotion may be derived from the motion through space, or "kinematics," of the limbs themselves, as well as that of their constituent segments.

Limb kinematics in particular have demonstrated significant applications to modeling gait dynamics. Walking involves an exchange between gravitational potential energy and kinetic energy as if the limbs are simple pendula (Cavagna et al. 1977). Running and hopping gaits differ from walking in that they incorporate the storage of elastic energy in the muscles and tendons, so for these gaits the limbs are better represented as springs attached to a central mass (Blickhan 1989; McMahon and Cheng 1990). The inverted pendulum and spring-mass models also reproduce experimental values for stride parameters and ground reaction forces (Geyer et al. 06). Both models represent the limb as a vector from endpoint (toe) to origin (hip). That a low degree of freedom variable such as a limb vector is sufficient to capture walking and running dynamics across an array of vertebrate and invertebrate taxa signifies that the limbs themselves may be important to the control of locomotion.

The significance of whole limb kinematics to vertebrate and invertebrate locomotion offer evolution-hardened biomechanical principles to the field of engineering. In this case, the inverted pendulum model of walking inspires conceptual designs of robots that walk stably down gentle slopes on rigid limbs powered only by gravity (Garcia et al. 1998; Coleman et al. 2001). Adding endpoint forces to the model that swing the limb at the hip and push off at the toe provides a more accurate depiction of walking dynamics (Kuo 2001; Kuo 2002). Both passive and powered walking robots built on these models walk as predicted (Collins et al. 2005). Similarly, principles of the spring-mass model inform the design of running robots (Ahmadi and Buehler 1997). The contributions of locomotor models to robotics underscore the potential significance of the limbs to motor control.

Yet limb kinematics may be more than a useful mathematical abstraction. The response of cat dorsal spinocerebellar tract (DSCT) neurons to hindlimb muscle

stimulation under mechanical constraints indicates that these neurons encode spatial information of limb axis length and angle (Bosco et al. 2000; Bosco and Poppele, 2000). These findings suggest that the central nervous system may integrate afferent feedback into internal representations of limb geometry (Poppele et al. 2002). If limb length and angle information exist at the spinal level before being transmitted to the cerebellum, this could mean that limb kinematics are involved in the spinal control of locomotion as well as supraspinal control.

1.2 Coordination of Locomotion

Modern investigation into the control of locomotion begins not with the brain but the spine. This originates from the discovery that the spinal cord produces the electrical stimulus controlling the continuous pattern of muscle flexion and extension associated with locomotion (Brown 1911). Networks of interneurons called central pattern generators (CPGs) generate these rhythmic motor signals independent of descending input from the brain (Grillner 1985). Traditional models of CPGs are based on half-center oscillators, paired neurons whose reciprocal inhibition facilitates an alternating sequence of flexion and extension (Pearson 1995). However the simple half-center model cannot explain certain experimental findings involving complex activity patterns, simultaneous regulation of timing and excitability, and fictive deletions (McCrea and Rybak, 2008). The half-center oscillator may be expanded into a two-tiered organization in which a rhythm generating level of interneurons controls a pattern formation level of interneurons, and these pattern formation networks are responsible for the activation of groups of motoneurons (Rybak et al. 2006). The precise organization of central pattern generators reflects the manner of control the neuromuscular system exerts over the body.

The supraspinal and peripheral nervous anatomy are also involved in the control of locomotion. Lesion and electrical stimulation experiments reveal many locomotor functions facilitated by supraspinal structures such as a descending motor drive that can

stimulate spinal CPG circuits from the brainstem, enhancement of muscle activation by the Deiters' nucleus and the red nucleus, refinement of motion by Purkinje cells of the cerebellum, and coordination of more skilled or demanding locomotion by the motor cortex (Armstrong 1988). Neuron activity in the motor cortex increases significantly when cats walked over the rungs of a horizontal ladder, a task which demands greater accuracy of paw placement (Beloozerova and Sirota 1993). Neuronal recordings also show that the motor cortex is involved in behaviors such as stepping over obstacles during locomotion, an adaptation triggered by visual rather than tactile stimuli (Drew 1993). The supraspinal structures together with the spinal cord define the central nervous system, but the peripheral nervous system is also involved in motor control. Proprioceptive input from cat ankle extensor muscles provides inhibition of limb flexion during stance phase (Duysens and Pearson 1980). Group Ia and Ib sensory nerve fibers regulate stance and swing duration through afferent feedback to the rhythm generating level of the central pattern generator (Pearson 1995). It is evident that motor control is an integrated effort of the central and peripheral nervous systems, but the distribution of physiological structures involved obfuscates the internal processes by which the nervous system coordinates movement.

The relation between movement and the bioelectrical signals that empower it, from neuron to muscle activity, reveals intrinsic qualities of motor control. Bernstein, father of modern motor control theory, deduced from equations of motion and muscle properties that a specific motor output could not be the result of a unique descending motor signal, but what must be instead "a system of impulses without unequivocal correspondence to the movement" (Bernstein 1967). He further reasoned that the coordination ("common action of separate elements") that produces movement is not the product of individual neurons, but rather the organized activity of a network of neurons. Patterns of muscle activity termed muscle synergies have been investigated as targets of this coordination that serve as modular building blocks in the control of behaviors such as

movement and posture (Ting and Macpherson 2005; Tresch et al. 2006; Ting and McKay 2007; Clark et al. 2010). Though much can be gleaned from the correlation of EMG activity with movement, muscle synergies do not necessarily represent the hypothesized control signals of movement.

A related approach is to analyze the coordination of kinematic outputs and from the properties of this organization derive insight into the nature of motor control. Dynamical systems theory posits that the elements of a system may be represented such that each element is one degree of freedom in a task-space with a defined task goal (Saltzman and Kelso, 1987). Under this theory, performance of the individual elements may vary yet performance of the task goal will persist if the elements coordinate to compensate for each other's deviations. In a system such as locomotion with its complex anatomical networks, there are more elements than necessary to achieve task goals; this property is described as equifinality or redundancy (Bernstein 1967, Latash et al. 2001). Incorporating engineering principles into the study of locomotion helps provide quantifiable predictions to hypotheses tackling the goals of motor control.

Application of dynamical systems theory to locomotion propagates notions of a hierarchical organization to motor control. Description of human movement as hierarchical has a long history, generally referring to the subsets of anatomical systems within systems (Turvey 2007). Many elements of the neuromuscular system fall under this paradigm: limbs, joints, muscles, neural networks, and individual neurons for example. The evident redundancy in structure within these subsystems suggests that the motor function of these elements may be coordinated in task space to perform task goals. The collective motions of each joint define limb kinematics; the net innervations of muscles crossing a joint establish the joint angle trajectory; a muscle's activity is the product of various combinations of motor units; the inhibitory and excitatory inputs from many axons influence the firing rate of a single motoneuron (Purves et al. 2004; Boron and Boulpaep, 2005; Prinz 2006). In each case, the motor function of a given element is

itself the coordinated response among the redundant motor functions of a lower level of anatomical organization. Given the hierarchical structure of motor redundancies as well as the hierarchical structure of locomotor CPGs, it's reasonable that motor control operates by principles shaped by these hierarchical attributes.

Application of dynamical systems theory to locomotion is limited in that, unlike engineering, there is a dearth of information on how the neuromuscular system defines task goals. The ultimate objectives of locomotion are transporting the center of mass through space while maintaining its equilibrium (Abbas and Full 2000; Dietz 2002). But the goals of other levels of motor control, and how these goals are represented and evaluated internally, remain a mystery. The stable kinematic trajectories of limbs and joints over the gait cycle qualify as performance variables (Latash 2010). It may not be possible to directly verify whether these variables also serve as actual internally regulated task goals or "implicit goals" of a particular hierarchical level. Nevertheless implicit goals may be indirectly assessed through experimental design. My approach is to investigate implicit goals of locomotion by evaluating the persistence of task goals in response to perturbations of a dynamical system. With it I evaluate my general hypothesis that locomotion is controlled through coordination of motor functions at a specific hierarchical level to maintain the implicit goals of a higher level.

1.3 Injury Compensation and Locomotion

Persistence in the face of change by a variable could be interpreted to represent its significance to the system, especially when that variable is the coordinated output of a subsystem. In evolutionary biology, convergent traits are those that are shared between organisms but not derived from a common heritage, from whence comes the saying, "What matters, converges" (Vogel 2003). This statement signifies that traits critical to organismal fitness resist the forces of evolutionary drift and manifest across diverse phylogenies. In locomotion, performance variables are considered "stabilized" through

invariance to perturbation over many gait cycles (Dingwell and Cusumano 2000). Humans stabilize limb motor function against cycle-to-cycle locomotor perturbations through the coordination of joint-level redundancies (Auyang et al. 2009; Chang et al. 2008; Ivanenko et al. 2007; Yen et al. 2009; Yen and Chang, 2010). Stability of performance variables against imposed perturbations may be evidence of the identity and character of implicit goals.

Surgically inducing injuries are a classic experimental perturbation based on the fundamental scientific principle of disabling part of a system to understand the function of the whole. Injury, that is damage to physiological tissues, has innumerable gradations of severity and permanence. Denervation of select ankle plantarflexor muscles is a common experimental injury model in cats (Whelan et al. 1995; Pearson et al. 1999; Bouyer et al. 2001; Pearson et al. 2003; Frigon and Rossignol, 2007; Donelan et al. 2009; Maas et al. 2010; Prilutsky et al. 2011). These studies often focus on changes in individual muscle activity of the intact plantarflexor over motor performance at the limb level. In studies of rat locomotion, nerve injuries typically occur at the spinal cord (Muir and Whishaw, 1999; Webb and Muir 2002; Webb and Muir 2003; Webb and Muir 2004; Webb and Muir 2005; Ballermann et al. 2006; Muir et al. 2007; Kanagal and Muir, 2008a; Kanagal and Muir, 2008b; Kanagal and Muir, 2009), sciatic nerve (Varejao et al. 2003), or tibial and peroneal nerves (Yu et al. 2001). Through this dissertation work I will expand the ankle plantarflexor denervation injury literature to include kinematics data of healthy rats and those with unilateral peripheral nerve injuries.

The variety of contexts against which the implicit goals of locomotion remain undisturbed is also unknown. Preservation of limb kinematics through coordination of joint kinematic redundancy may be a task goal that extends from cycle-to-cycle or transitive situations to more chronic perturbations of neuromuscular system function, such as after peripheral nerve injury. Cats with self-reinnervated ankle plantarflexor muscles demonstrate intralimb coordination that stabilizes pre-injury limb parameters

(Chang et al. 2009). This suggests stabilization of pre-injury limb kinematics may have some universal presence across taxa, injury, and environment. I will extend the examination of the persistence of limb kinematics as a task goal of locomotion to rat peripheral nerve injury models (Chapter 3).

In the clinical context associated with injury, preservation of limb kinematics could be termed an injury compensation strategy. By definition "compensation" suggests change by lower-level elements to make up for a deficit among other elements in order to sustain a higher goal. Many mechanical and dynamic characteristics of gait compensation are shared across taxa and pathologies. The most ubiquitous is limping or antalgic gait. Limping may be quantitatively expressed as asymmetry in stance and swing durations between bilateral limb pairs (DeVisser et al. 2005; Sawyer and Kapoor, 2009). If preservation of limb kinematics and limping are two common features of injury compensation, then this raises questions of which implicit goals the intact or contralateral hindlimb is following and whether preservation of pre-injury bilateral limb symmetry is a task goal of the interlimb level of the hierarchy. If the intact limb were to continue to follow the pre-injury task goal of limb kinematics preservation, then the subject seemingly cannot limp as well, as this would produce limb motion symmetrical to the injured hindlimb. I will extend the examination of the persistence of limb kinematics as a task goal of locomotion to the intact hindlimb rat peripheral nerve injury models in order to address the question of whether the intact limb deviates from this pre-injury task goal (Chapter 4).

Experimental manipulation of a subject's external environment is another basic scientific technique to study the response of biological systems to applied perturbations. For instance, changing the surface grade or slope of locomotion influences limb loading conditions. Walking upslope requires more work to be performed against gravity than walking over a level surface. Quadrupeds walking up an incline exhibit increases in EMG magnitude and duration compared to level walking (Pierotti et al. 1989, Carlson-Kuhta et

al. 1998), as well as greater peak ground reaction forces (Gregor et al. 2006; Lammers et al. 2006) and ankle moments (Maas et al. 2009). Conversely, decline locomotion demands relatively less work against gravity. Quadrupeds walking downslope demonstrate smaller EMG magnitudes (Smith et al. 1998), peak forces (Gregor et al. 2006; Lammers et al. 2006), and ankle moments (Maas et al. 2009). Altering the surface grade is a non-invasive, modular, bilateral locomotor perturbation with innumerable experimental applications.

By combining the bilateral perturbation of slope walking with the unilateral perturbation of these peripheral nerve injury models, I intend to experimentally modulate the behavioral expression of locomotor compensation (Chapter 4). Physiological impairment resulting from denervation entails the permanent loss of functional innervation to the targeted muscles, as well as the removal of afferent sensory feedback from the muscles (Pearson et al. 1999; Navarro et al. 2007). When these deficits occur unilaterally, we may expect that the discrepancies in motor and sensory functional capacity between hindlimbs to result in differing locomotor compensations to the same changes in limb loading. Specifically I expect limping will increase in response to upslope walking and decrease with downslope walking. Manipulating gait asymmetry will shed light on the preservation of pre-injury bilateral limb symmetry as a potential implicit goal of locomotion at the interlimb level of the locomotor hierarchy.

1.4 Significance

Rodents are critically important scientifically, yet current methods to assess locomotion are insufficient to measure significant details of limb motor function. Rats and mice comprise over 97% of all animals involved in biomedical research in the United States (Anon. 1986, Anon. 2001). Gross differences between normal and pathological gaits can be detected with the Sciatic Function Index and BBB locomotor ratings scales, qualitative surveys designed to quantify gait recovery after sciatic nerve and spinal cord

injury, respectively (Bain et al. 1989; Basso et al. 1995). However both assays have shown susceptibility to type II error, incorrectly indicating recovery when gait deficits were still present (Metz et al. 1998; Metz et al. 2000; Varejao et al. 2001; Varejao et al. 2003). Accurate limb and joint kinematics data is difficult to obtain from rats due to skin movement error (Muir and Webb, 2000). This occurs when the physical markers attached to the skin above each joint center do not match the actual position of the corresponding anatomical landmarks due to the inertial properties of the loose rat skin. I will demonstrate the capacity of X-ray technology to overcome these inherent limitations by comparing rat hindlimb kinematics data from high-speed X-ray video to that derived from skin markers (Chapter 2).

I leverage the increased accuracy of X-ray kinematics to study two peripheral nerve injury models in rats. One surgery involves denervation of all four major ankle plantarflexor muscles (medial and lateral gastrocnemius, soleus, and plantaris), henceforth the "severe" injury group, and another in which I denervate three of the four muscles but spare medial gastrocnemius, which I label the "moderate" injury group (Chapter 3). Comparing the stability of compensation across different degrees of injury provides context for the generalizability of limb kinematics as performance variables of locomotion. These findings could help explain how locomotor compensation fits into the larger puzzle of hierarchical motor control.

The purpose of my dissertation is to elucidate the implicit goals governing locomotion. My general hypothesis is that locomotion is controlled through coordination of motor functions at a specific hierarchical level to maintain the implicit goals of a higher level. The groundwork is laid in a methodological study of joint kinematics from a healthy rat limb (Chapter 2). Accurate rodent kinematics demand an advance in measurement technology. I then induce peripheral nerve injuries in rats to study *intralimb* coordination patterns (Chapter 3). I use these injury models to test the hypothesis that preservation of pre-injury limb kinematics is an implicit goal of locomotion. I continue

my examination of the hierarchical organization of motor control with *interlimb* coordination in the same injury models (Chapter 4). I introduce an additional experimental perturbation, slope walking, to address the hypothesis that preservation of pre-injury bilateral symmetry of limb kinematics is an implicit goal of a hierarchical level higher than that of individual limb kinematics. These three sets of experiments are represented schematically in Figure 1-1 in terms of the kinematic variables to be measured.

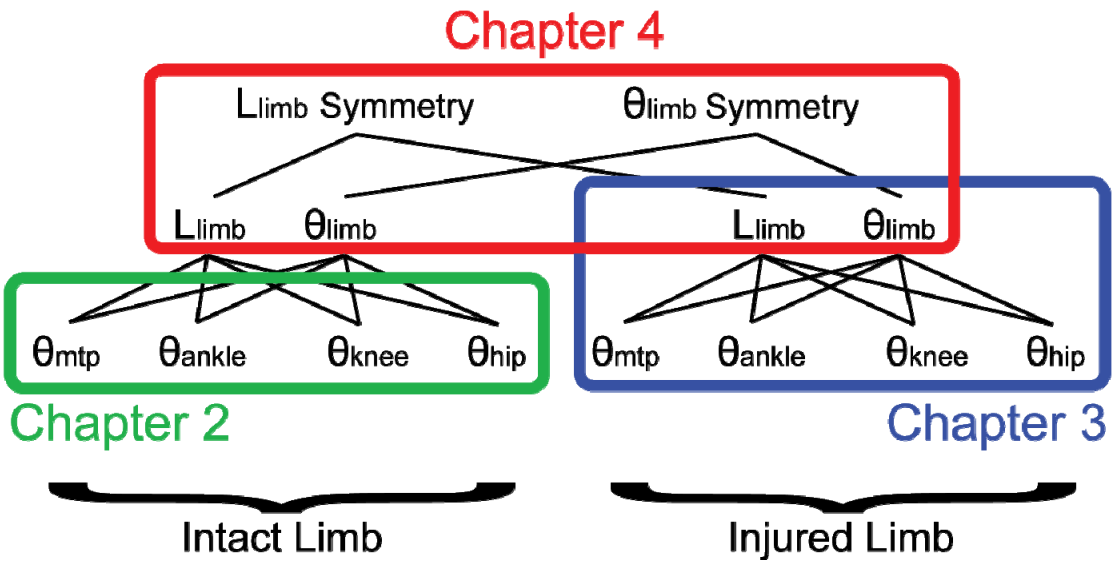

Figure 1-1. Schematic of experiments represented by kinematic variables
Kinematic variables from three different anatomical levels are arranged according to their hierarchical organization. The joint angle trajectories (θmtp, θankle, θknee, θhip) together with fixed limb segment lengths (not pictured) wholly define the length and angle trajectories of their respective limb (Llimb, θlimb). Analogously, individual hindlimb parameters are compared to quantify bilateral symmetry of limb kinematics.

Beyond expected insight into how implicit goals operate at multiple hierarchical levels of motor control, the results of this dissertation work will offer broad applications to associated scientific disciplines. For the utility of rat peripheral nerve injury models to match their prolificacy, there must be a systematized mapping of locomotor compensation patterns to specific nerve injuries. Understanding compensation to

permanent denervation of specific muscles will provide a basic framework for future studies that examine more complex injury models such as self-reinnervation, as well as prevalent higher order injury models such as sciatic nerve and spinal cord injury. A more realistic depiction of the internal motor control processes will improve models of locomotion used in industries such as animations and robotics. These results could improve clinical gait rehabilitation through future protocols that address restitution of motor patterns of the entire limb over those of individual joint trajectories, as well as inform the design of rehabilitation robotic walkers.

CHAPTER 2

HIGH SPEED X-RAY VIDEO DEMONSTRATES SIGNIFICANT SKIN MOVEMENT ERRORS WITH STANDARD OPTICAL KINEMATICS DURING RAT LOCOMOTION

This chapter was originally published in the *Journal of Neuroscience Methods*: Bauman JM and Chang YH. High-speed X-ray video demonstrates significant skin movement errors with standard optical kinematics during rat locomotion. *Journal of Neuroscience Methods* 186: 18-24, 2010.

2.1 Introduction

Rats and mice constitute the overwhelming choice for animal research models in the United States as approximately 97% of all animals used for biomedical research (Anon. 1986; Anon. 2001). With the wide range of disease models developed for biomedical research as well as the continued rise of genomic methods, the rat will likely continue to be relied upon as a model organism for studying rehabilitation of the central and peripheral nervous system after injury. Yet, it is questionable whether the accuracy of the joint kinematics methodologies currently available is sufficient to evaluate future injury and disease models. The detection of behavioral differences resulting from the subtle changes in motor output that often accompany different injury models demands more accurate and comprehensive measurement techniques.

Two widely used methodologies for quantifying rat locomotor behavior are the Sciatic Function Index (Bain et al. 1989) and the BBB locomotor rating scale (Basso et al. 1995), designed to evaluate recovery following sciatic nerve and spinal cord injuries, respectively. Although these continue to be excellent measures for gross behavioral differences, Metz and colleagues have recommended supplemental assays as they found

small but important behavioral changes between sham and pyramidal tract lesion rodents, yet equal BBB scores (Metz et al. 1998; Metz et al. 2000). Other investigators have also recommended more quantitative tests to analyze gaits among which more qualitative tests could not discriminate (Ballermann et al. 2006). For example, Varejao et al. (Varejao et al. 2001; Varejao et al. 2003) observed gait deficits in ankle kinematics after peripheral nerve injury despite SFI results indicating a full recovery. Even with ankle kinematics alone, no inferences can be drawn regarding the neuromuscular function of the majority of the limb muscles that cross the more proximal limb joints or any effects on intralimb compensation and coordination.

The major impediment to the adoption of whole limb kinematics in rats is the inaccuracy of joint kinematics measurements due to skin movement errors. Skin movement is acknowledged to be an important source of error in human kinematics (Capozzo et al. 1996; Alexander and Andriacchi, 2001). In rats, soft tissue of the rat hindlimb moves even more freely relative to the underlying limb bones, resulting in a significant magnitude of error in the proximal limb joints (Muir and Webb, 2000). Previous whole limb kinematic analyses in the rat have either neglected to account for skin error, or utilized a triangulation algorithm that assumes an improved estimate of knee joint position (Filipe et al. 2006). In this study we tackle the question of whether the obstacle of skin movement error can be overcome in rat kinematics measurement.

The purpose of this study was to simultaneously compare kinematics methods based on direct skeletal tracking ("bone-derived kinematics") to those originating from markers attached to the skin ("skin-derived kinematics"). Any differences from the bone-derived kinematics in the simultaneously measured skin-derived and triangulated kinematics methods would then provide the errors due to skin and soft tissue movement. Direct visualization of the appendicular skeleton during locomotion provides the best possible estimate of hindlimb joint centers without any influence from skin movement artefacts (Fischer et al. 2002). This is typically achieved with X-ray technology. Our

central hypothesis was that bone-derived kinematics would differ from skin-derived kinematics.

Another goal of this study was to simultaneously compare bone-derived kinematics to a variant of skin-derived kinematics that features triangulated kinematics. The concept of triangulation is intended to mitigate skin movement error by estimating the position of the knee joint center rather than relying on an attached skin marker for that one point (Chang et al. 2008, 2009). We hypothesized that bone-derived kinematics would differ from triangulated kinematics, but to a lesser degree than purely skin-derived kinematics.

The final objective of this study was to examine the factors that influence skin movement error in order to determine when skin-derived markers could yield accurate kinematics. We traced skin movement errors over a range of speeds to evaluate the contribution of dynamic limb movements as well as across the range of static limb postures exhibited over a gait cycle. Our last hypotheses were that the magnitude of skin movement error for each joint would increase with greater treadmill speeds and at the extremes of the limb angle trajectory.

2.2 Methods

Using high-speed X-ray video analysis, we simultaneously recorded bone-derived and skin-derived positions of anatomical landmarks from the same stride cycles to quantify errors that were due only to skin movement. We also triangulated the knee joint center from the skin markers as an additional third kinematics method for comparison to bone-derived kinematics. This experimental design inherently eliminated any inter-trial variability such that all differences in joint angle measurements were due solely to differences between the three tested kinematics methods, which we refer to as: (i) bone-derived; (ii) skin-derived; and (iii) triangulated.

2.2.1 Animal care and training

We tested six adult male Sprague-Dawley rats (average weight 250.74 ± 33.30 g) in accordance with a protocol approved by the Georgia Institute of Technology IACUC. Each animal was prepared for experimental trials by shaving the fur from the left hindquarters and hindlimb while under isoflurane gas anesthesia (5% induction, 2-3% maintenance). The rats were held in a prone position, supported beneath the torso with the hindlimbs positioned on the floor below the body such that the MTP joint was vertically aligned with the hip joint. Care was taken to maintain the rats in this state approximating a mid-stance limb posture during locomotion to make the best possible correspondence between skin-derived and bone-derived markers. Six anatomical hindlimb features were marked with permanent ink after careful identification through manual palpation: the 4th distal phalanx, 4th metatarsal head, lateral malleolus of the fibula, lateral epicondyle of the femur, greater trochanter, and the caudal margin of the ischium (Fig. 2-1). After the positions were verified and corrected as necessary, 0.8 or 1.0 mm radio-opaque tantalum spheres (RSA Biomedical) were glued to the marked skin with cyanoacrylate gel.

2.2.2 Data collection

During experiments the animals were enclosed in a 21.5 x 15.0 x 56.0 cm Plexiglas treadmill (Rat Modular Treadmill, Columbia Instruments) oriented perpendicular to the beam of a custom-built high-speed X-ray video system (Fig. 2-2). X-rays were emitted by a continuous beam X-ray emitting tube (97 kV, 2 mA, Monoblock-160, VJ Technologies). The photons passed through the animal and into a 225 mm diameter input window image intensifier (TH-9438-HX, VJ Technologies) where they were converted to visible light (548 nm wavelength) and recorded by a high-speed digital video camera (A504k, Basler Vision Technologies). All data were recorded at 200 Hz and saved using commercial software (Streampix, Norpix, Inc.) to a dedicated computer.

We constructed a 2-D calibration frame consisting of lead beads ordered in a square grid pattern (1 cm apart) on a Plexiglas sheet to autocorrect for any image distortion that may

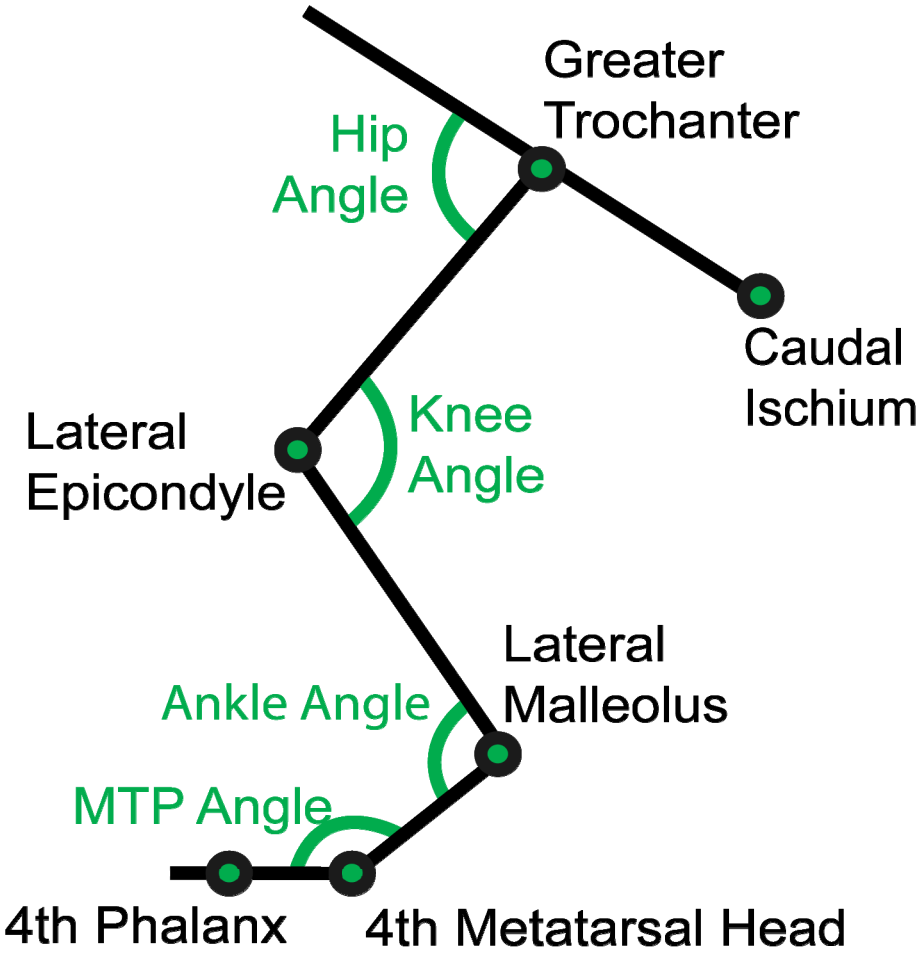

Figure 2-1. Sagittal plane kinematic model of rat hindlimb
Six tantalum markers (filled circles) were placed over bone landmarks corresponding to (proximal to distal): caudal ischium, greater trochanter, lateral epicondyle of the femur, lateral malleolus of the fibula, fourth metatarsal head, and the fourth distal phalanx. Included joint angles for hip, knee, ankle and metatarsophalangeal joints along with limb angle were calculated.

have occurred. A CNC mill was used to mark the placement of the lead beads to ensure accuracy of bead placement to within 0.00254 mm. Commercial image correction software (NI Vision software, National Instruments) was used to correct video data against the calibration grid.

Each rat was acclimated to the treadmill at a sampling of different speeds one week prior to the first experimental trial. A single data collection trial consisted of a ten

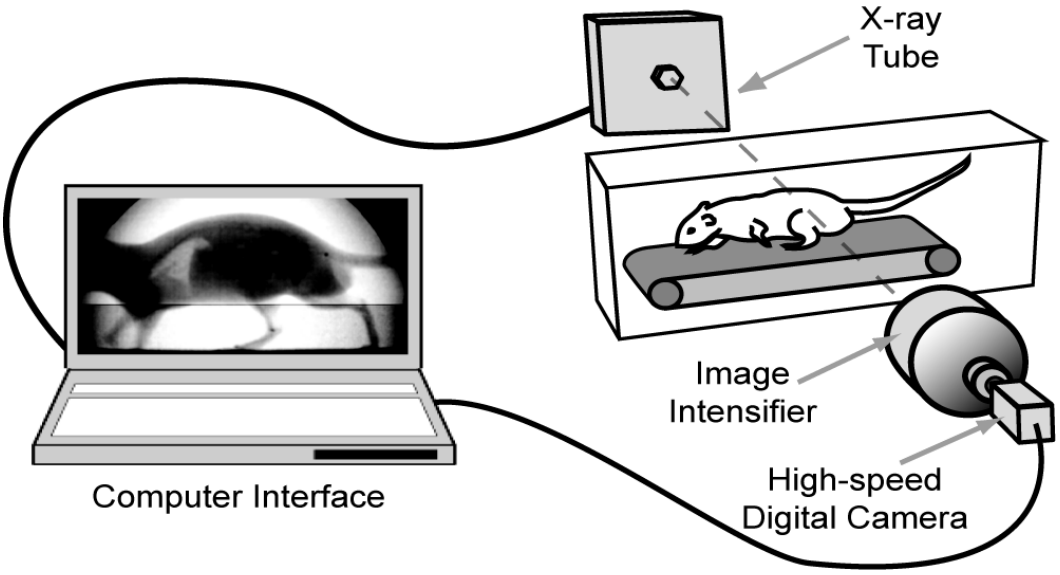

Figure 2-2. Schematic of experimental set-up
Equipment required to collect X-ray kinematics include a custom x-ray tube, treadmill, image intensifier, high-speed digital camera, computer interface, and an animal subject.

second X-ray exposure while the rat locomoted steadily at a constant speed. An entire day's session was composed of 10-12 trials over a range of treadmill speeds (16.5-63.2 cm/s), which were adjusted to the animal's capabilities for that day. Each rat participated in three data collection sessions over the course of approximately 14 days.

2.2.3 Data processing

After data collection, we visually assessed videos for steady locomotion and cropped them into shorter clips containing at least three consecutive strides. Data clips contained 8.94±7.70 strides with a range of 3-30 strides across all animals. We defined a stride as one ipsilateral paw contact to the successive ipsilateral paw contact. We then exported the clips as sets of still images for the purposes of X-ray image distortion correction and contrast enhancement, which were accomplished using commercial image analysis software (NI Vision software, National Instruments). We then reconstituted the

individual images back into a video file for digitization. We separately image enhanced and digitized each clip depending upon the different tracking targets. In this way we maximized contrast and identification of skeletal landmarks for the bone-derived kinematics and the tantalum markers for the skin-derived kinematics. The radio opaque tantalum spheres demonstrated sufficient contrast to be automatically tracked with an open source MATLAB software (DLTdataviewer, Tyson Hedrick, UNC-Chapel Hill). The bony landmarks were manually digitized through frame-by-frame inspection and identification with visual reference to an articulated rat skeleton.

In addition to direct digitization of the knee position via the attached skin marker (i.e., skin-derived kinematics), we also estimated knee position through triangulation (i.e., triangulated kinematics), another common optical kinematics technique (Chang et al. 2008, 2009). The inputs for the triangulation method included the sagittal plane skin-derived positions of the hip (greater trochanter) and ankle (lateral malleolus) as well as the averaged femur and shank lengths (measured from X-ray video data). We then triangulated the position of the knee joint center by finding the two points of intersection between two circles with centers defined by the ankle and hip joint markers and radii defined by the length of the respective femoral and tibial limb segments, of which only one solution is anatomically viable. Note that the results of the triangulation method share with the skin-derived measurements the positions of all joint centers except the knee, however, a difference in knee position will affect the calculation of all three joint angles.

We transformed the three sets of estimated joint position data into corresponding sets of sagittal plane included joint angles using custom MATLAB code. Hip angle was defined by the supplement of the angle formed by the knee joint center, greater trochanter, and caudal point on the ischium (Fig. 2-1). Knee and ankle joint angles were defined by the smaller angle formed by the joint in question and the adjacent proximal and distal joint positions. We time-normalized all step cycles to one hundred percent of the stride cycle beginning with paw contact on the ground.

We performed student's t-test on the mean kinematics trajectory data at every 1% of the gait cycle to test for statistical differences of the bone-derived kinematics data against skin-derived kinematics and triangulated kinematics. We used α=0.10 in situations with small sample sizes and α=0.05 for all other cases. All t-tests and regression analyses were performed with MATLAB software.

2.3 Results

For each animal, we pooled all step cycles from all trials at a given speed to generate a single representative mean trajectory for that animal and condition. We then calculated mean kinematics trajectories for each of 12 treadmill speeds across all animals, as well as for all cycles (n=237) from all speeds pooled together.

2.3.1 Locomotor Kinematics

Stick figure reconstructions of the hindlimb kinematics data show clear qualitative differences in the limb configurations estimated by the three kinematics methodologies at key times during the stride (Fig. 2-3). All joint positions calculated with skin-derived or triangulated methods indicated some differences with respect to bone-derived kinematics. Estimates of knee position were particularly poor compared to hip and ankle. We observed striking differences in the estimated knee positions at the instant of paw contact.

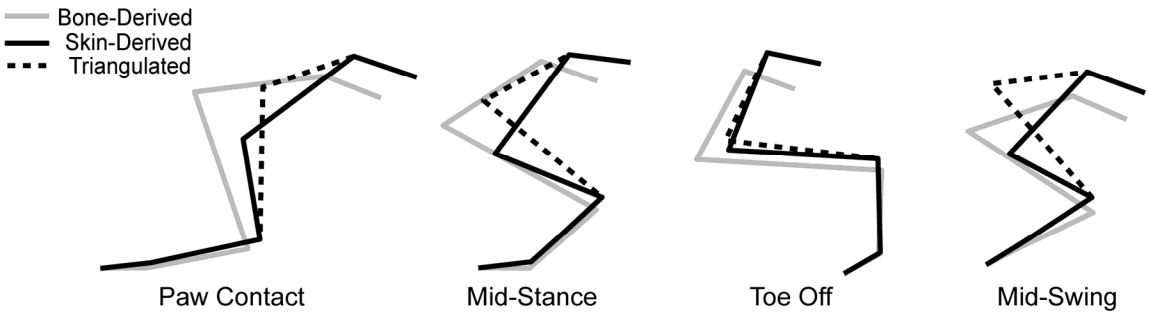

Figure 2-3. Stick figures of the hindlimb throughout the gait cycle
Average joint center positions were calculated from bone-derived (gray), skin-derived (solid black) and triangulated (dashed) kinematics methods. Four specific times during

the stride cycle are shown for four animals walking at 40 cm/s. Data are averaged over 44 gait cycles.

The differences in results between methodologies are best illustrated with kinematics (Fig. 2-4) from the same representative animals as the previous figure. For all three joints the largest difference between skin and bone-derived angles always occurred at the time period immediately before paw contact. At that moment the magnitudes of greatest error were 31±22º for the hip ($p<<0.001$), 39±6º for the knee ($p<<0.001$), and 14±6º for the ankle ($p<<0.001$). These t-tests were performed with ($\alpha=0.10$) because of the sample size (n=4 rats). Skin-derived hip angle values indicated a greatly reduced hip range of motion across the stride cycle. Skin-derived kinematics exaggerated knee range of motion, and lacked the inflection point observed with bone-derived kinematics during late stance. Skin-derived ankle kinematics most closely resembled bone-derived kinematics compared to the knee and hip data. Triangulated kinematics appeared to have smaller differences from bone-derived kinematics than skin-derived kinematics for the hip and knee joints, but there was still a peak knee angle difference of 17±11º near paw contact ($p<<0.001$). The ankle joint is the one case where skin-derived kinematics are a closer approximation of bone-derived kinematics than are those of triangulation

The magnitudes and durations of differences compare to bone-derived kinematics, or "error," for both skin-derived and triangulated kinematics over the gait cycle (Fig. 2-5) was considerable. Skin marker and triangulation errors were each significant ($p<0.05$) for over 80% of the gait cycle for the hip joint, but skin maker error tended to overestimate while triangulation error underestimated bone-derived kinematics. Both skin marker and triangulation errors were biased toward over estimation for knee joint angle kinematics. Triangulated ankle angle error was significant for the entire stride, while skin maker error had a mixed pattern despite being significant for the majority of the cycle. At this exemplary speed of 40 cm/s, skin-derived and triangulated kinematics differed

significantly from bone-derived kinematics for no less than 76% of the gait cycle. For every speed surveyed ($p<0.10$), skin marker error was present for at least 50% of the gait cycle.

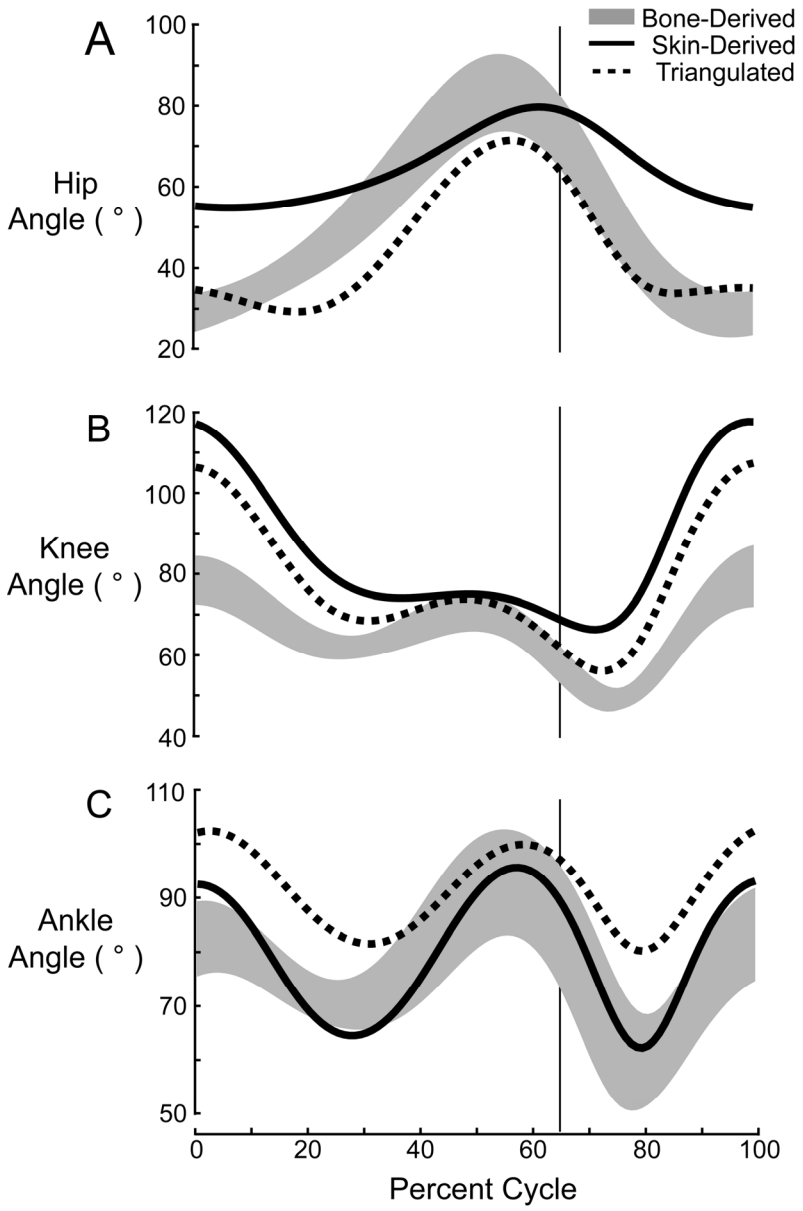

Figure 2-4. Joint kinematics methodology comparison
Mean kinematics for the hip (A), knee (B), and ankle (C) joints were calculated from bone-derived (gray±1SD), skin-derived (solid) and triangulated (dashed) kinematics methods for the same representative animals as in Figure 2-3. Vertical line indicates end of stance phase and beginning of swing phase.

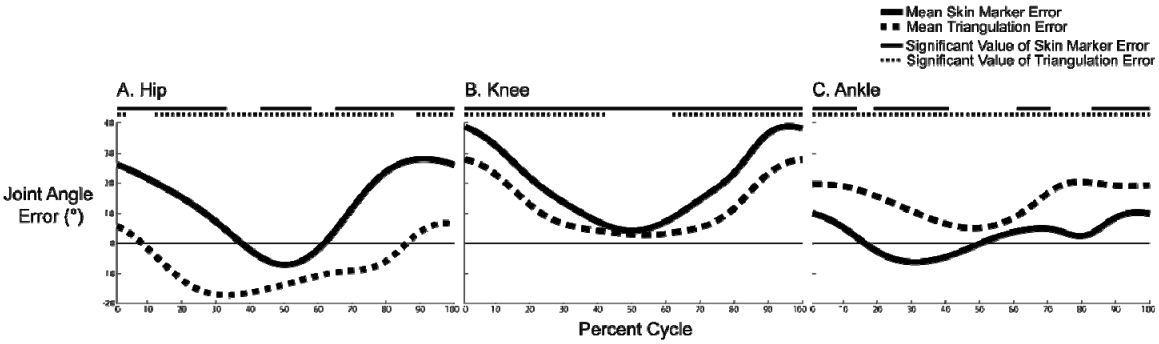

Figure 2-5. Joint angle error comparison

Mean joint angle errors for the hip (A), knee (B), and ankle (C) joints were calculated from the differences between skin and bone-derived (solid) and between triangulated and bone-derived (dashed) kinematics methodologies for the same representative animals as Figures 2-3 and 2-4. T-tests (α=0.05) were calculated at every 1% of the normalized gait cycle and denoted with a thin line across the top when the error was a significant difference between methodologies.

2.3.2 Sources of Skin Movement Error

Comparing the joint center accelerations measured by bone-derived and skin-derived kinematics provided insights into the dynamic effects of the inertial properties of the skin and soft tissue during locomotion. We calculated the second-derivative of the resultant root mean square (RMS) positions to obtain sagittal plane joint center accelerations for the knee and hip joints (Fig. 2-6), which are the joints with the most soft tissue movement. The skin-derived method overestimated peak joint center acceleration and deceleration for both joints compared to bone-derived joint center accelerations. The skin-derived knee joint center acceleration trajectory also displayed an important divergence from the bone-derived data in late stance phase. Bone-derived knee joint

center acceleration had a bimodal trajectory, whereas the skin-derived trajectory had a unimodal trajectory (Fig. 2-6A).

Peak joint center accelerations decreased with increasing treadmill speed (Fig. 2-7). Skin-derived methodologies overestimated peak accelerations. In a similar trend, joint angle errors calculated at paw contact (Fig. 2-8) decreased slightly with increasing

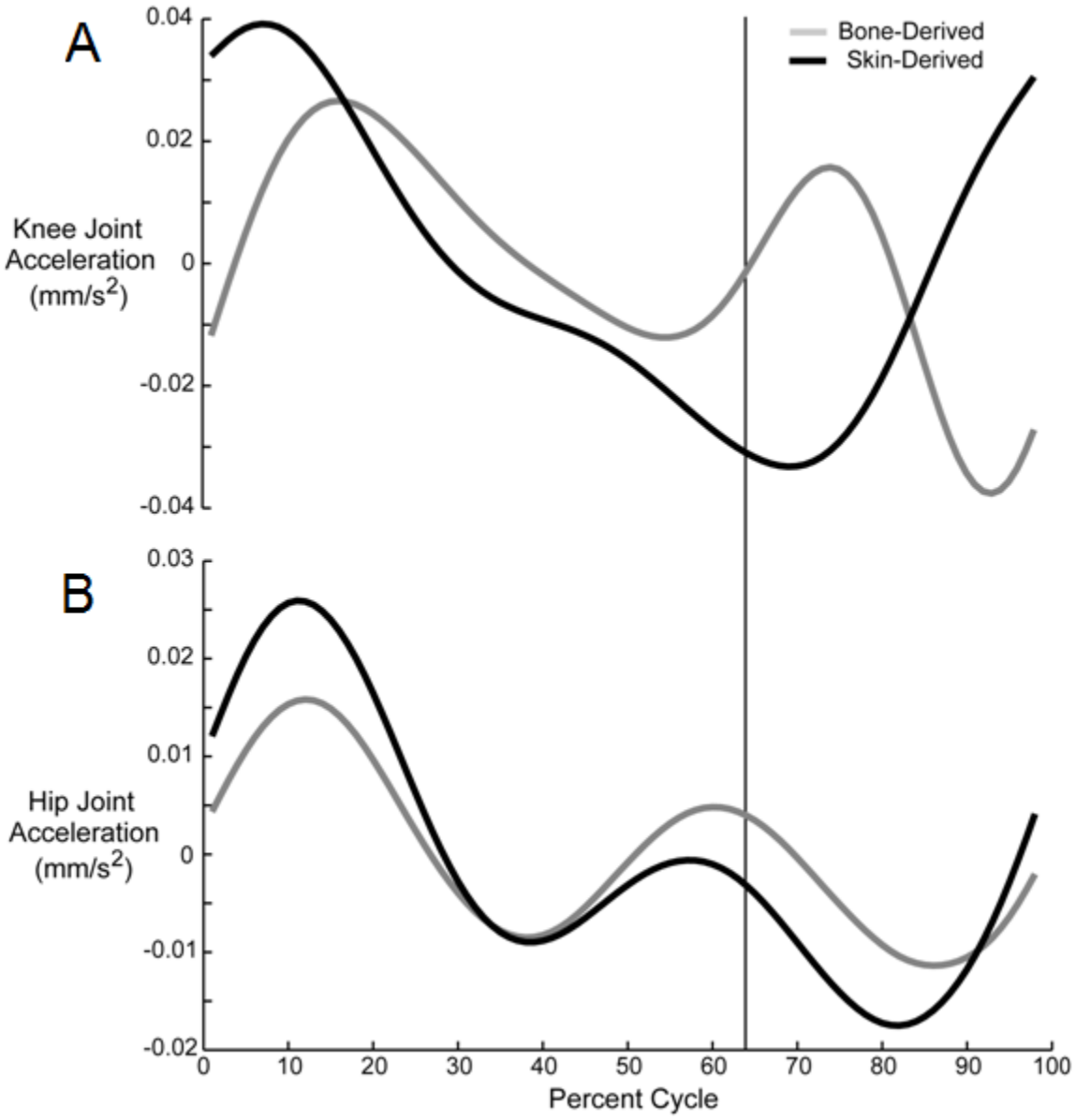

Figure 2-6. Joint center acceleration vs. gait cycle comparison
Mean resultant knee (A) and hip (B) joint center trajectories calculated from both skin-derived (black) and bone-derived (gray) methods pooled for all rats across all speeds. Vertical line indicates transition from stance to swing.

treadmill speed. These parameters represent separate glimpses at the relationship between skin movement error and locomotor speed, as the peak joint center accelerations do not necessarily occur around the paw contact event.

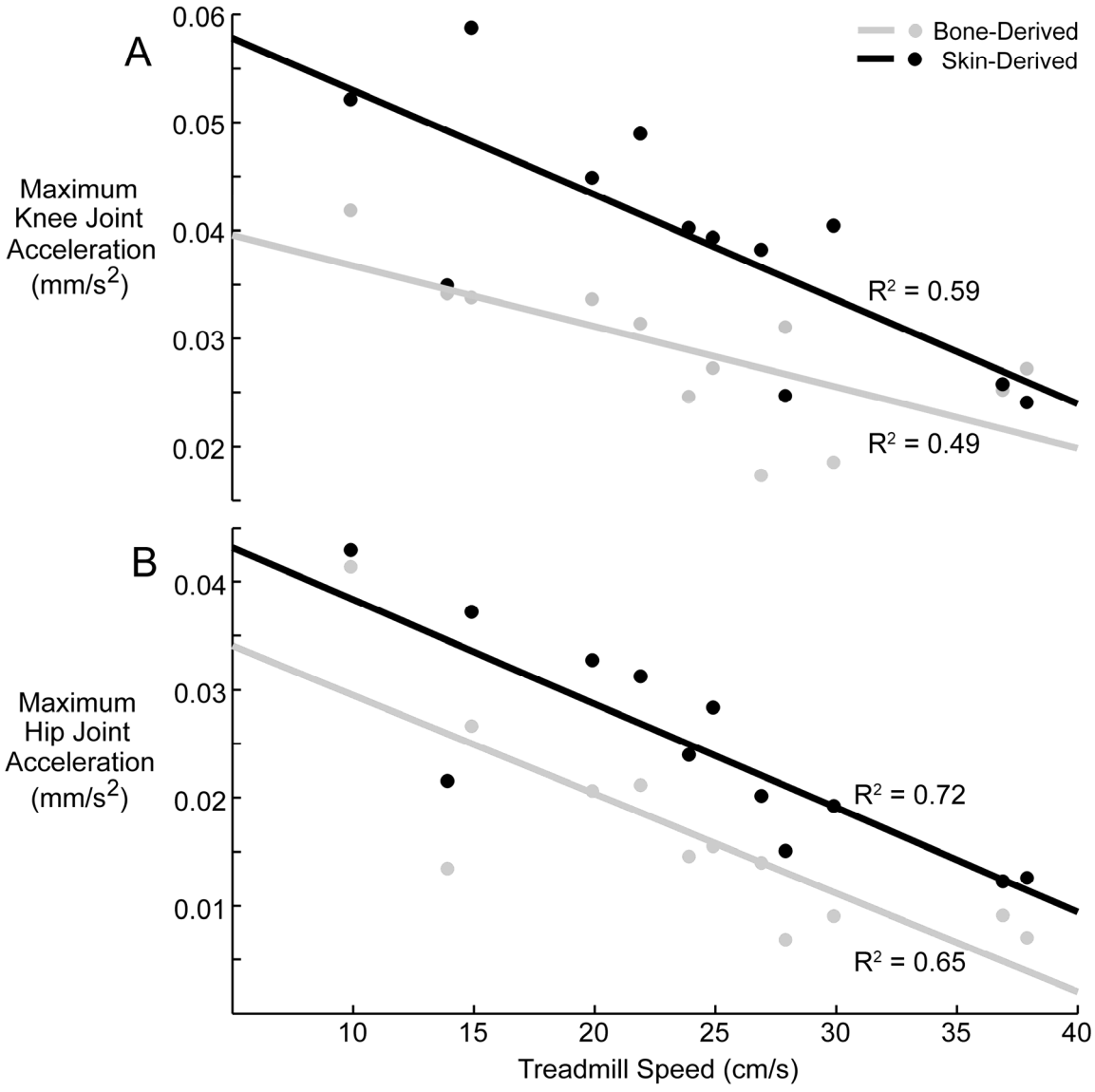

Figure 2-7. Joint center acceleration vs. speed comparison
Maximum knee (A) and hip (B) joint acceleration values calculated from both skin-derived (black) and bone-derived (gray) methods as a function of treadmill speed. Each symbol represents the average of all animals tested at that particular speed.

We further investigated the significance of skin movement through errors in estimated joint center positions as a function of limb posture (Fig. 2-9). Knee joint positions calculated by both methods had the least amount of error when the hindlimb was most flexed, or the posture adopted near the end of stance phase when the toe is coming off of the ground. We saw the greatest knee position errors for both methods

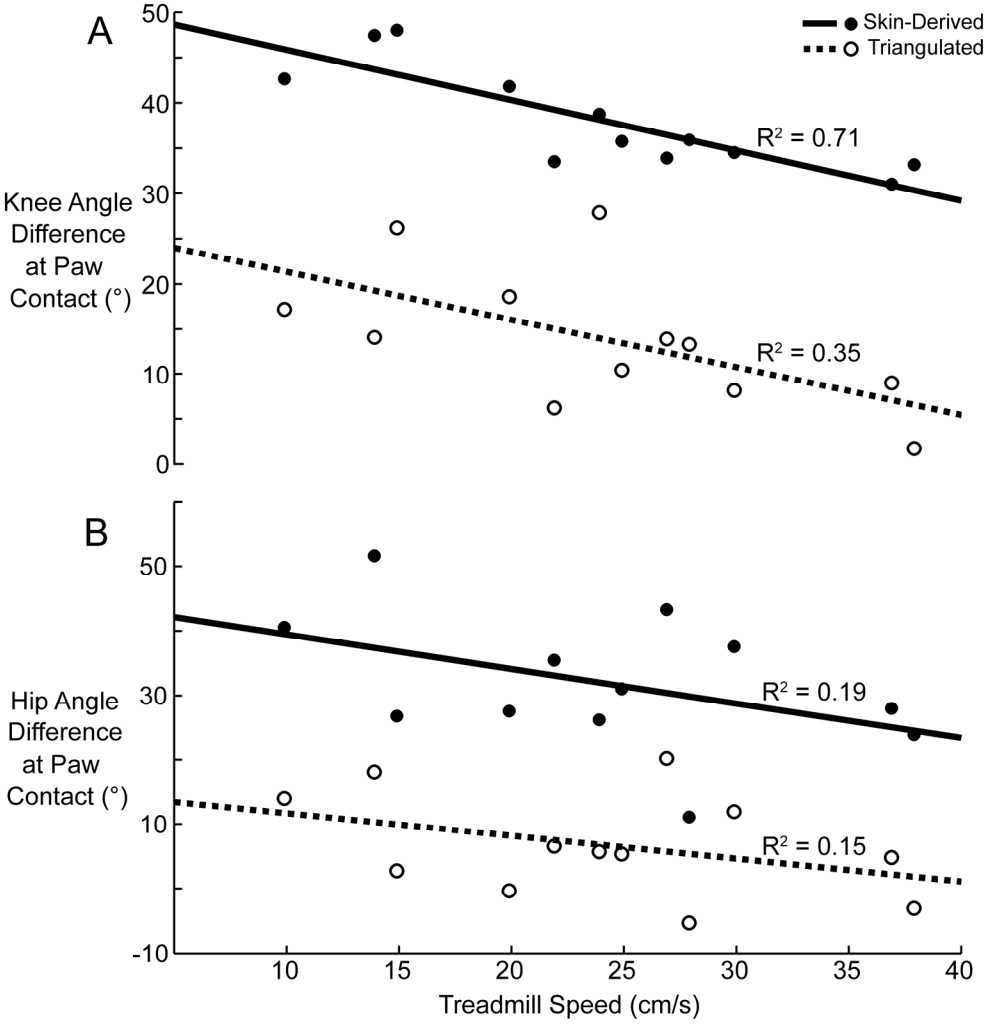

Figure 2-8. Kinematic error versus treadmill speed
Knee (A) and hip (B) joint angle error at foot contact as a function of treadmill speed due to skin-derived (solid) and triangulated (dashed) kinematics methods. Joint angle error was defined as the difference with respect to bone-derived kinematics. Each symbol represents the average of all animals tested at that particular speed.

occurring when the limb was most extended, near the beginning of stance phase when the paw first contacts the ground. Although the skin-derived method had greater errors than the triangulated kinematics, they both showed similar trends with limb orientation angle.

These position errors due to soft tissue movement and kinematics methodology resulted in substantial effects on a common measure for characterizing intralimb coordination: angle-angle plots. The knee-ankle coordination estimated by both skin-derived and triangulated kinematics methods grossly misrepresented the ranges of movements of both joints as well as the characteristic shape of the intralimb coordination pattern compared to calculation from actual skeletal movements (Fig. 2-10).

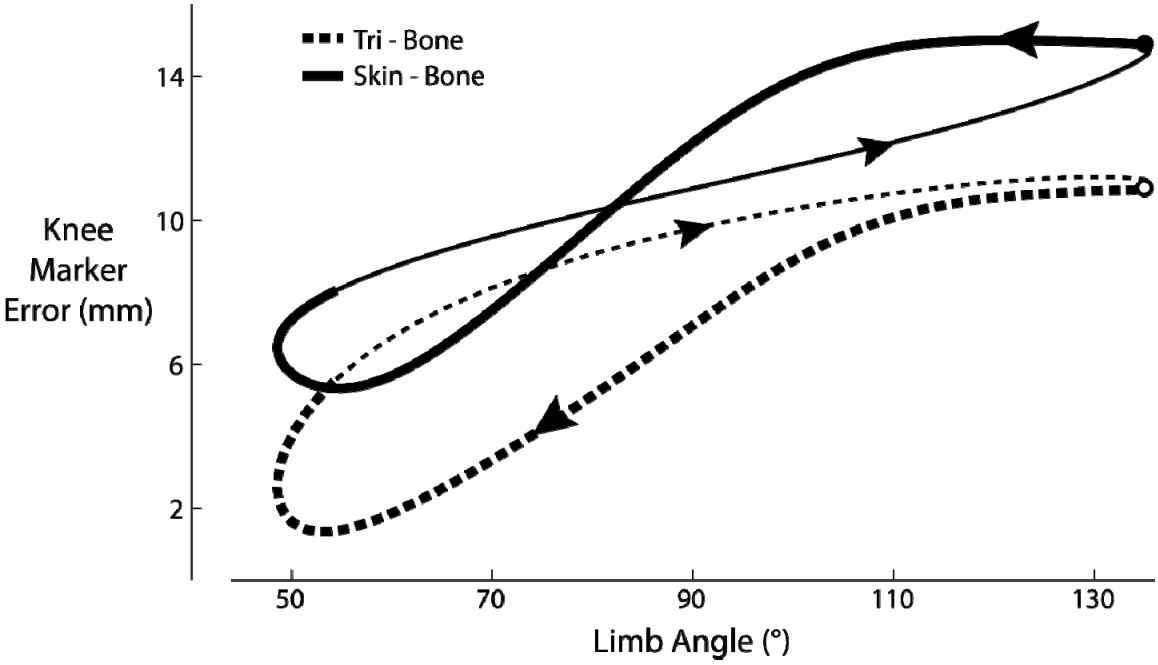

Figure 2-9. Root mean square error in knee marker position
Root mean square (RMS) error in knee joint position estimated from skin-derived (solid) and triangulated (dashed) methods as a function of limb orientation. Mean trajectories are pooled from all six rats across all treadmill speeds. Circles represent paw contact and the beginning of stance phase (thick lines) and end of the swing phase (thin lines). Arrows indicate flow of time over the stride cycle.

2.4 Discussion

The main goal of this study was to quantify and compare the joint kinematics calculated over a range of locomotor speeds using three different methodologies. Since our experimental design ruled out inter-trial variability by using the exact same step cycles to compare each method, any differences can be attributed to errors due to skin and soft tissue movement. As those differences were often significant, we made a primary assumption that kinematics from bone-derived joint positions were the most accurate of the three due to the direct identification of joint centers from skeletal

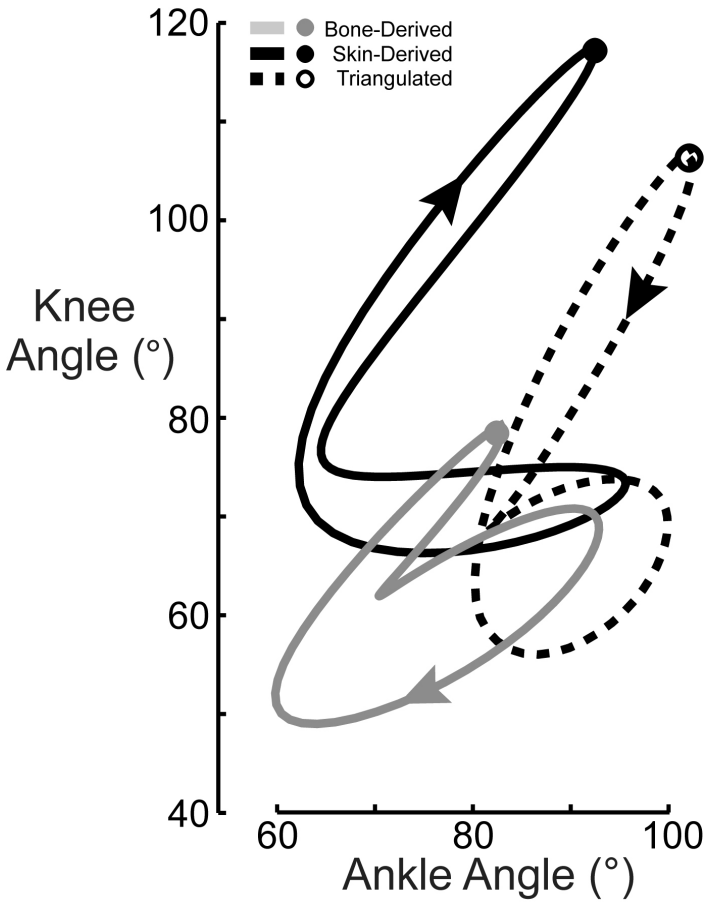

Figure 2-10. Knee-ankle coordination
Mean coordination patterns of the knee joint angle as a function of ankle joint angle calculated from bone-derived (gray), skin-derived (solid black) and triangulated (dashed) kinematics methods for four animals walking at 40 cm/s. Circles represent paw contact. Arrows indicate flow of time over the stride cycle.

landmarks, which bypassed influences from all soft tissues (Fischer et al. 2002). The inter-cycle variability for the bone-derived kinematics was consistent with the other two

methods (average SD = 4-7º for all three methods), suggesting that the digitizing process was consistent across strides for all three methods.

While detailed rat hindlimb kinematics have been limited to only a handful of studies, our results are consistent with respect to previous findings for each of the three respective methodologies. Our skin-derived joint angle trajectories are consistent with published results of rat hip (Bennett et al. 2012; Filipe et al. 2006; Pereira et al. 2006) and knee (Bennett et al. 2012; Filipe et al. 2006; Gillis and Biewener, 2001; Pereira et al. 2006; Thota et al. 2005) kinematics. Our triangulated knee joint angles were also similar to previously published knee kinematics using the triangulation method (Filipe et al. 2006). Kinematics from all three joints of our bone-derived kinematics agree to within 10º of the only other limited description of X-ray kinematics in a common laboratory rat (R. norvegicus, (Fischer et al. 2002)). However, our sample size was larger as that study reported data from only one cycle from one animal.

The magnitudes of our ankle angles were slightly smaller for the skin-derived and triangulation methodologies throughout the stride cycle than those in the published literature (Filipe et al. 2006). This is most likely due to a more cranially estimated knee position in our skin-derived and triangulated data, which in our study actually better matched the bone-derived measurements. Ankle joint kinematics were actually worse with the triangulation method compared to the skin-derived method with values overestimating ankle joint angle.

The skin-derived hip and knee joint angles were significantly different from those of bone-derived marker positions for much of the gait cycle across all speeds (Fig. 2-5). We accept our initial hypothesis that the magnitudes of joint angle errors due to skin-derived kinematics are greater in the proximal knee and hip joints compared to the more distal ankle joint. Skin-derived kinematics, particularly that of the knee joint at paw contact, can be exceedingly inaccurate. In addition, the amount of joint kinematics error changes over the stride cycle, with treadmill speed, and general limb posture such that a

simple systematic baseline correction is not of any use. Therefore, any use of skin-derived joint kinematics in rats (and presumably mice and other small mammals) should be done with great caution since the knee and hip angles could be overestimated by as much as 39° and 31°, respectively.

Joint kinematics calculated with the triangulation method were generally more accurate than the skin-derived kinematics (Fig. 2-5). By comparison, the triangulated knee and hip joint trajectories are more accurate over much of the stride cycle, often staying within one standard deviation of the mean bone-derived kinematics. The error measured in ankle joint angle produced by a triangulated knee position, however, is often larger than the errors in knee and hip across all speeds. Therefore, we reject our first hypothesis for triangulated kinematics.

Our secondary objective was to analyze potential sources of the skin movement errors. We considered both inertial (dynamic) and postural (static) components to skin movement error. Our second hypothesis predicted that joint kinematics errors would increase with treadmill speed and also with more extreme ranges of limb posture. This was partially predicated on the notion that greater limb segment velocities generated during faster locomotion would increase inertial movements of the skin, and thereby increase the error between skin and bone-derived kinematics. Surprisingly, we found that the opposite occurred (Fig. 2-8). There was a slight decrease in joint kinematics errors with locomotion at greater treadmill speeds. Both skin-derived and bone-derived peak joint accelerations unexpectedly decreased with speed, so we reject our second hypothesis with respect to the increased errors due to inertial effects at faster speeds. This phenomenon could be due to increased tension in the skin resulting from the increased rate of limb cycling. It is worth noting that the greater peaks and the difference in trajectory shapes of the skin-derived and bone-derived accelerations support the notion that the dynamics of soft tissue movements are different from those of the skeleton (Figs. 2-6, 2-7).

Limb posture may be the single greatest determinant to explaining the accuracy of skin-derived kinematics. The errors in knee joint center positions reached a minimum at the most protracted limb angle for both skin-derived and triangulated methods. This protracted limb posture corresponds to the end of the stance phase, where the minimum joint angle differences can be seen to occur. This also corresponds to the most extended postures for each individual joint, which likely helps to minimize the effects of any errors in joint center positions. Conversely, maximum errors for both skin and triangulation-derived knee positions occurred just before paw contact, which also corresponds with each of the joints being in generally flexed postures. However, the relationship between knee marker error and limb angle is not strictly linear due to the hysteresis of the trajectory (Fig. 2-9). For triangulated kinematics the error is always greater during swing phase than stance phase, whereas for skin-derived kinematics the relative error between stance and swing phases varies with limb posture. These results imply that other unknown factors are necessary to fully explain the nonlinear pattern of skin movement errors across the stride cycle. Nevertheless, there is a good correlation of knee error with the toe-to-hip limb orientation for both kinematics methods.

Skin movement error is particularly exacerbated at early stance, mitigated in late stance, and may be slightly attenuated at higher walking speeds. Our observation that errors are minimized around toe off corresponds with some recommendations in the literature for the gait events at which sciatic and peripheral nerve injuries should be evaluated (Lin et al. 1996; Yu et al. 2001). Again, however, this advice should be taken with some ambivalence since there is currently no standardized method for selecting the limb posture at which skin markers should be attached. This practically precludes the recommendation of a strict skin movement error correction algorithm, as the trajectories of the errors may depend on the initial marker placement. Correspondingly, an interesting area for further investigation would be to study the effects of changing the limb posture at

which skin markers are attached to see if it is possible to target specific portions of the gait cycle where accurate kinematics could be attained.

Accurate kinematics are a prerequisite for higher-level neuromechanical analyses such as those requiring the characterization of intralimb coordination patterns or the calculation of joint torques generated during locomotion. We have shown, for example, that the intralimb coordination pattern between the knee and ankle joints can appear to be quite different depending solely on the kinematics method used (Fig. 2-10). Quantification of intralimb coordination can be an important tool for characterizing subtle changes in locomotor control during recovery from nerve injury. As we show, the errors associated with non-bone-derived kinematics can lead to very misleading conclusions about how the joints are being controlled and coordinated during locomotion. The bone-derived kinematics associated with X-ray videography currently yields the most accurate kinematics measurements in rodents. Fortunately, this is a technology that is becoming increasingly common and accessible to researchers in a growing number of fields from comparative biomechanics (de Groot et al. 2004; Druzisky and Brainerd, 2001; Fischer et al. 2002; Jenkins, 1972; Jenkins, 1970; Jenkins et al. 1988a, b; Snelderwaard et al. 2002), orthopedic biomechanics (Banks et al. 2005; Fregly et al. 2005; Tashman and Anderst, 2003; You et al. 2001), and neuroscience (Boczek-Funcke et al. 1994; Boczek-Funcke et al. 1999; Boczek-Funcke et al. 2000; Graf et al. 1995; Kuhtz-Buschbeck et al. 1996; Rabbath et al. 2001; Vidal et al. 2004). To our knowledge, this is the first systematic use of high-speed fluoroscopy to quantify limb movements in standard laboratory rats.

There are some drawbacks to the choice of X-ray fluoroscopy. Like other biomechanics methodologies, data analysis may demand a large amount of manual digitization with some opportunity for automatic tracking. Admittedly, our X-ray fluoroscopy system is a technological compromise while remaining forward-looking. It is more advanced than traditional optical video, but not as sophisticated as several 3-D X-

ray kinematics systems that currently exist. A wider range of hypotheses involving pathological rodent gait models could be tested with joint kinematics from three planes. Our system provides accurate sagittal plane kinematics, however 3-D kinematics may be necessary to describe true flexion-extension angles if significant out-of-plane motion is suspected. State-of-the-art biplanar fluoroscopy systems already in use permit measurement of 3-D kinematics as well as automatic bone tracking based on CT measurements (Dawson et al. 2009, Fahrig et al. 1997; Keefe et al. 2008; Li et al. 2006; Tashman and Anderst, 2003; Tashman et al. 2004; You et al. 2001). Furthermore, existing techniques also provide the opportunity for best-fitting accurate 3-D skeletal models derived from CT scans onto 2-D X-ray images (Banks et al. 2005; Fischer et al. 2001; Fregly et al. 2005)).

2.5 Conclusions

We have demonstrated the significant differences between bone-derived kinematics and typical optical kinematics techniques: skin-derived and triangulated measurements. We determined that triangulated kinematics are closer to bone-derived kinematics that are skin-derived kinematics for the knee and hip joint angles, but not ankle angle. We further found that increasing locomotor speed has a slight attenuating effect on some of these errors, but that much of the errors can be attributed to the soft tissue artefacts associated with the general orientation of the limb. Although relatively few investigators currently use high-speed X-ray kinematics for neuroscience research, given the continued importance of rats and mice as research models for human disease these techniques are likely to become a standard wherever high precision or great accuracy is required in a behavioral assay.

CHAPTER 3

CONSERVATION OF SINGLE LIMB FUNCTION AFTER PERIPHERAL NERVE INJURY

3.1 Introduction

Simple biomechanical models can robustly predict a wide range of locomotor behaviors. The dynamics of walking and running gaits across an array of vertebrate and invertebrate taxa may be predicted from low degree of freedom behavioral templates, such as a spring-loaded inverted pendulum (Cavagna et al. 1977; Blickhan, 1989; McMahon and Cheng, 1990). The robustness of these models is based on the consistency of limb behaviors from stride to stride. Neurophysiological studies also suggest whole limb kinematics are physiologically relevant and meaningful parameters for legged locomotion. Recordings from cat dorsal spinocerebellar tract neurons provide evidence that the central nervous system integrates afferent feedback into neural representations of whole limb angle and length (Bosco and Poppele, 2000; Bosco et al. 2000; Poppele et al. 2002). Together these lines of research suggest that limb behaviors are critical aspects of locomotion.

Redundant configurations of joint angles are coordinated by the neuromuscular system to define limb kinematics. The classical motor control problem of redundant degrees of freedom (Bernstein, 1967) may be a solution to maintain consistent limb behavior across varying locomotor conditions. Recent studies have shown humans utilize joint-level redundancies to stabilize limb function with respect to cycle-to-cycle locomotor perturbations (Ivanenko et al. 2007; Chang et al. 2008; Auyang et al. 2009; Yen et al. 2009; Yen et al. 2010). The deficits in joint function with injury can be viewed

as a reduction in redundancy of the system. We addressed the research question of how intralimb coordination maintains locomotion after the loss or redundancy due to injury.

Common experimental injury models in rats may be inappropriate to isolate limb behaviors. Compensatory locomotor behaviors have been assessed after spinal cord and sciatic nerve injuries, which create deficits in motor and sensory function throughout the hindlimb (Muir and Webb, 2000; Varejao et al. 2001). A more controlled perturbation of limb behavior would be to surgically denervate one or more muscles at a single hindlimb joint. During the paralytic stage after self-reinnervation of cat ankle extensor muscles, the ankle joint exhibits increased flexion while the hip and knee joints are more extended (Maas et al. 2007). Yet whole limb kinematics are conserved through the coordinated changes across all joints of the injured limb (Chang et al. 2009). We wished to further examine the general hypothesis that post-injury joint angle combinations are selected to achieve consistency, or a minimum change, in limb length and angle during legged locomotion.

We tested this hypothesis by evaluating limb kinematics after permanent denervation of specific combinations of rat ankle plantarflexor muscles. We hypothesized that limb kinematics after peripheral nerve injury in rats would correlate highly with pre-injury limb measurements. We expected the subjects to compensate for the loss of ankle joint function with coordinated changes of all hindlimb joints. To that end we further hypothesized that post-injury joint kinematics would assume fundamentally new trajectories.

3.2 Methods

Twenty-three adult male Sprague-Dawley rats (Rattus norvegicus) were housed in pairs and provided food and water ad libitum. Peripheral nerve injuries were induced in 19 rats under aseptic conditions and isoflurane gas anesthesia. Two types of interventions were performed: a “severe” injury that denervated or permanently paralyzed all four

major ankle plantarflexor muscles, and a "moderate" injury that denervated only lateral gastrocnemius, soleus, and plantaris but left medial gastrocnemius intact. We chose to spare medial gastrocnemius largely due to the extensive history of this type of surgical intervention in cats, and secondarily because of the greater anatomical difficulty in isolating the nerves to lateral gastrocnemius, soleus, and plantaris (Whelan et al. 1995; Pearson et al. 1999, Maas et al. 2007; Chang et al. 2009; Donelan et al. 2009). Incisions were made in the posterior popliteal fossa of each left hindlimb and branches of the tibial nerve leading to the medial and lateral gastrocnemius, soleus, and plantaris muscles were exposed. The nerve branches were transected at their proximal most and distal most branch points and an approximate 5-10 mm section was removed. Denervation of target muscles was visually verified by electrical stimulation to the proximal portion of the tibial nerve before wound closure. Muscles were stimulated again during the terminal surgery to further confirm denervation as well as to verify the absence or reinnervation. We performed all surgical procedures in accordance with the Georgia Institute of Technology IACUC.

The moderate (n=10 rats) and severe (n=9) surgery groups participated in kinematic data collection at least seven days post-injury. Some members of the control group (n=15) later underwent surgery as part of a separate repeated measures design study (Chapter 4); here they are treated as independent subjects. Each rat was acclimated to treadmill locomotion prior to surgery. The animals were enclosed within a Plexiglas treadmill (Columbia Instruments) oriented perpendicular to the beam of a custom, high-speed X-ray video system (Bauman and Chang, 2010). A single trial consisted of 10 seconds of X-ray exposure while the rat locomoted steadily at 0.4 meters/second. Given an average hindlimb length of approximately 0.12 meters, this speed was equivalent to a Froude number of 0.14, which is safely below the value of 0.5 that would signal transition from a walk to another gait (Alexander 2003). A data collection session yielded 10-12 trials per rat.

Joint and limb kinematic data were extracted from sagittal plane X-ray videos. Video data were first corrected for distortion and enhanced for contrast (NI Vision, National Instruments). Hindlimb features were manually digitized frame-by-frame using open source MATLAB software (Hedrick, 2008). Marker position data were low-pass filtered (7 Hz) and used to derive limb and joint kinematics by the convention of Figure 3-1. Six anatomical landmarks (4^{th} distal phalanx, 4^{th} metatarsal head, lateral malleolus of the fibula, lateral epicondyle of the femur, greater trochanter, and the caudal margin of the ischium) defined four included joint angles [metatarsophalangeal (MTP), ankle, knee, and hip joints]. The limb kinematic vector was defined from the 4^{th} distal phalanx marker to that of the greater trochanter. Each stride was time-normalized to one hundred percent of the gait cycle. Mean kinematics trajectories were calculated for each animal, and then averaged across subjects to report grand mean trajectories for each experimental condition.

We used linear regression as a goodness-of-fit measure to statistically compare shapes of kinematics trajectories between the control and injured groups. For each kinematics parameter, we calculated coefficients of determination (R^2) comparing each injured rat trajectory to the mean control group trajectory over the entire normalized gait cycle. We then performed a t-test of the hypothesis that the mean R^2 value across individual rats for each injury condition would explain the majority of the variance of limb and joint kinematics parameters of the control rats, i.e. mean R^2 will be significantly greater than 0.5. We choose this 0.5 threshold as the standard for similarity of two trajectories because it represents when one trajectory explains greater than fifty percent of the variance in another trajectory (Chang et al. 2009). Applying this measure to fit a nonlinear model to experimental data can result in coefficients of determination outside the standard [0,1] interval (Cameron and Windmeijer 1997). This includes potentially negative values of R^2, which in the context of goodness-of-fit signifies that the mean of

the data would fit the model better than the actual data distribution. All statistical analyses were performed with MATLAB software.

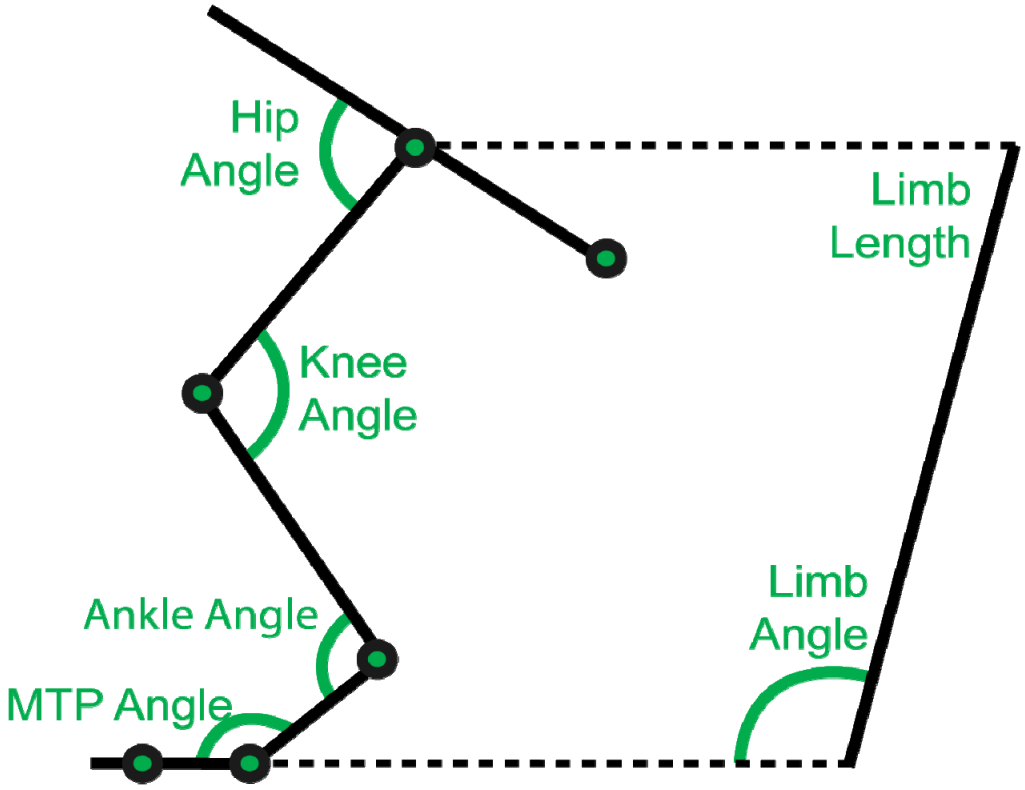

Figure 3-1. Sagittal plane kinematic model of rat hindlimb
Six tantalum markers (filled circles) were placed over bone landmarks corresponding to (proximal to distal): caudal ischium, greater trochanter, lateral epicondyle of the femur, lateral malleolus of the fibula, fourth metatarsal head, and the fourth distal phalanx. Included joint angles for hip, knee, ankle and metatarsophalangeal joints were calculated. The limb vector was measured from the fourth distal phalanx to the greater trochanter and defined limb length and angle.

3.3 Results

We collected kinematic data at one, two, three, and four weeks post-injury. Cats with equivalent peripheral nerve injuries have been tested as soon as five hours after the anesthesia from surgery wears off (Pearson et al. 1999). We chose to measure kinematics no sooner than one week post-injury to focus on long-term compensation mechanisms to

permanent injury. Subsequent analysis found no differences in kinematics between time points, so the data is presented with all time points pooled together.

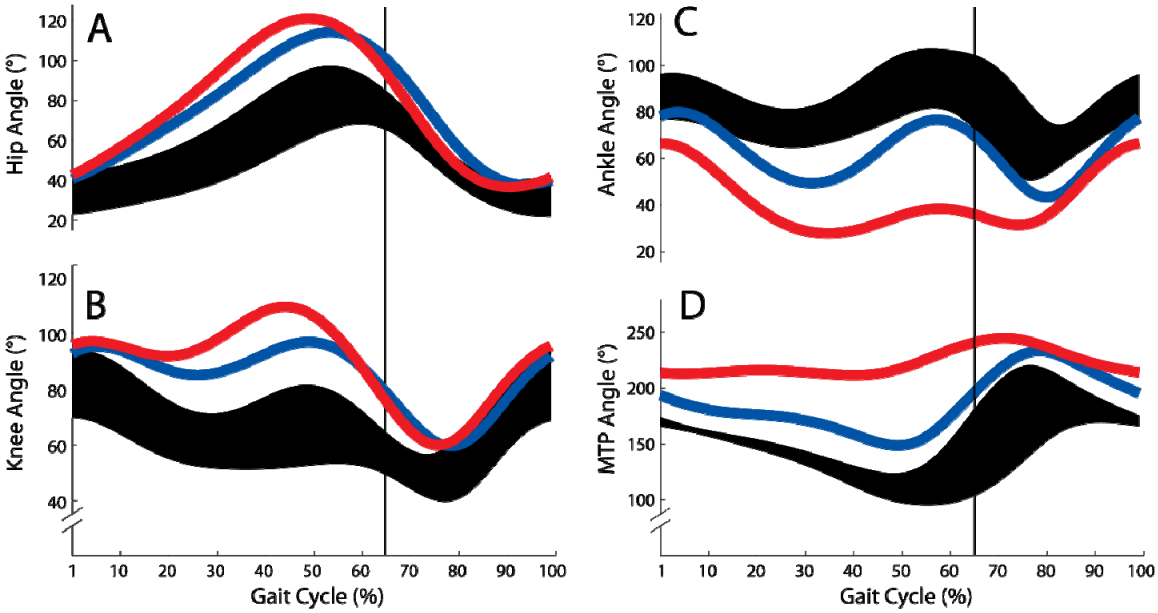

Figure 3-2. Joint kinematics after peripheral nerve injury
Hip (A), knee (B), ankle (C) and MTP (D) trajectories were plotted over a normalized gait cycle. Control data (black, n=15) was represented by ± 1 S.D. about the mean and compared to mean trajectories after moderate (blue, n=10) and severe (red, n=9) triceps surae denervations. The vertical bars represent the transition between the stance and swing phases of gait.

Both injury interventions disrupted pre-injury ankle behaviors. Mean joint kinematics after surgical denervation of ankle extensor muscles fell outside the range of one standard deviation of mean control trajectories (Fig. 3-2). The trend of changes was consistent across experimental groups: the ankle joint was more yielding or flexed during post-injury locomotion, whereas the hip, knee, and MTP joints were all more extended than those of the control group. The correlation between control and post-injury joint kinematics (Table 3-1) confirmed that pre-injury trajectories were significantly different than post-injury trajectories. No joint-level comparison had an average R^2 value greater

Table 3-1. Average coefficients of determination (R^2) relating control and post-injury kinematics

$R^2 = 1$ signifies complete correspondence between data sets. $R^2 < 0$ signifies that the mean value of post-injury kinematics over the gait cycle is a better fit to the control data than the trajectory itself. T-tests verified whether post-injury kinematics explained greater than 50% of the variability of control data ($p<0.05$).

	Severe PNI	Moderate PNI
Limb Angle	$R^2 = 0.82 \pm 0.09$ ($p<0.001$)*	$R^2 = 0.91 \pm 0.02$ ($p<0.001$)*
Limb Length	$R^2 = 0.69 \pm 0.08$ ($p<0.001$)*	$R^2 = 0.61 \pm 0.10$ ($p=0.005$)*
Hip Angle	$R^2 = 0.35 \pm 0.18$ ($p=0.981$)	$R^2 = 0.57 \pm 0.17$ ($p=0.160$)
Knee Angle	$R^2 = -2.38 \pm 0.83$ ($p=1.000$)	$R^2 = -1.49 \pm 1.04$ ($p=1.000$)
Ankle Angle	$R^2 = -7.20 \pm 2.22$ ($p=1.000$)	$R^2 = -2.10 \pm 1.78$ ($p=0.999$)
MTP Angle	$R^2 = -26.04 \pm 19.14$ ($p=0.998$)	$R^2 = -0.32 \pm 0.85$ ($p=0.993$)

than 0.5. The magnitude of changes, however, depended on the extent of injury, as denervation of all four muscles ("severe" injury) tended to produce more deviation from baseline joint kinematics than when the medial gastrocnemius was left intact ("moderate" injury).

In contrast, the limb kinematics vector was conserved after peripheral nerve injury. Mean limb angle (Fig. 3-3A) and limb length (Fig. 3-3B) trajectories for both the severe and moderately injured rats fell within one standard deviation of mean control

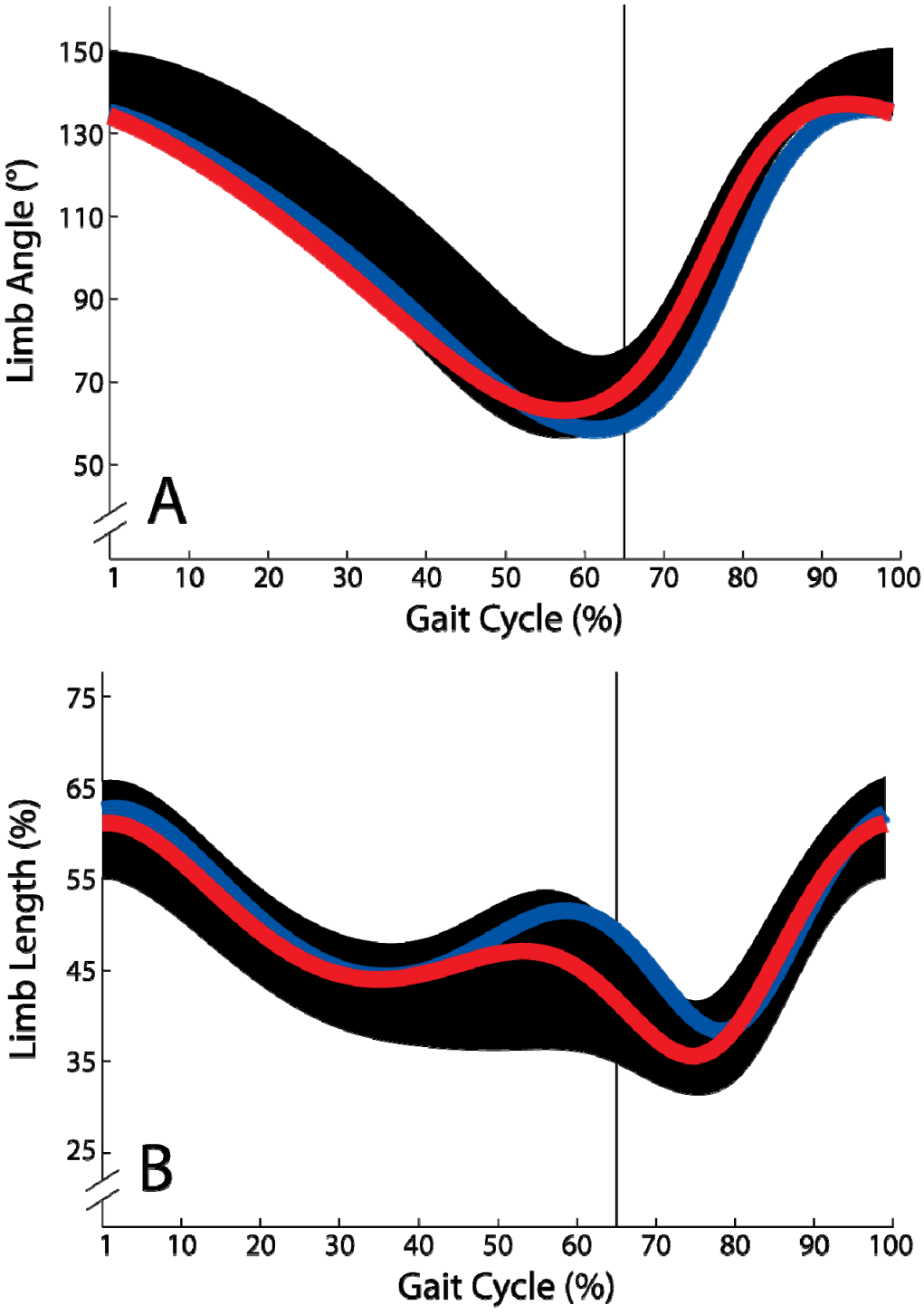

Figure 3-3. L**imb kinematics after peripheral nerve injury**
Changes in mean limb angle (A) and limb length (B) after peripheral nerve injury. Data follows the same conventions as Figure 3-2.

trajectories. Correlation between control and post-injury limb kinematics concluded that the trajectories matched, as mean R^2 values for limb angle and length were all significantly greater than 0.5 (Table 3-1).

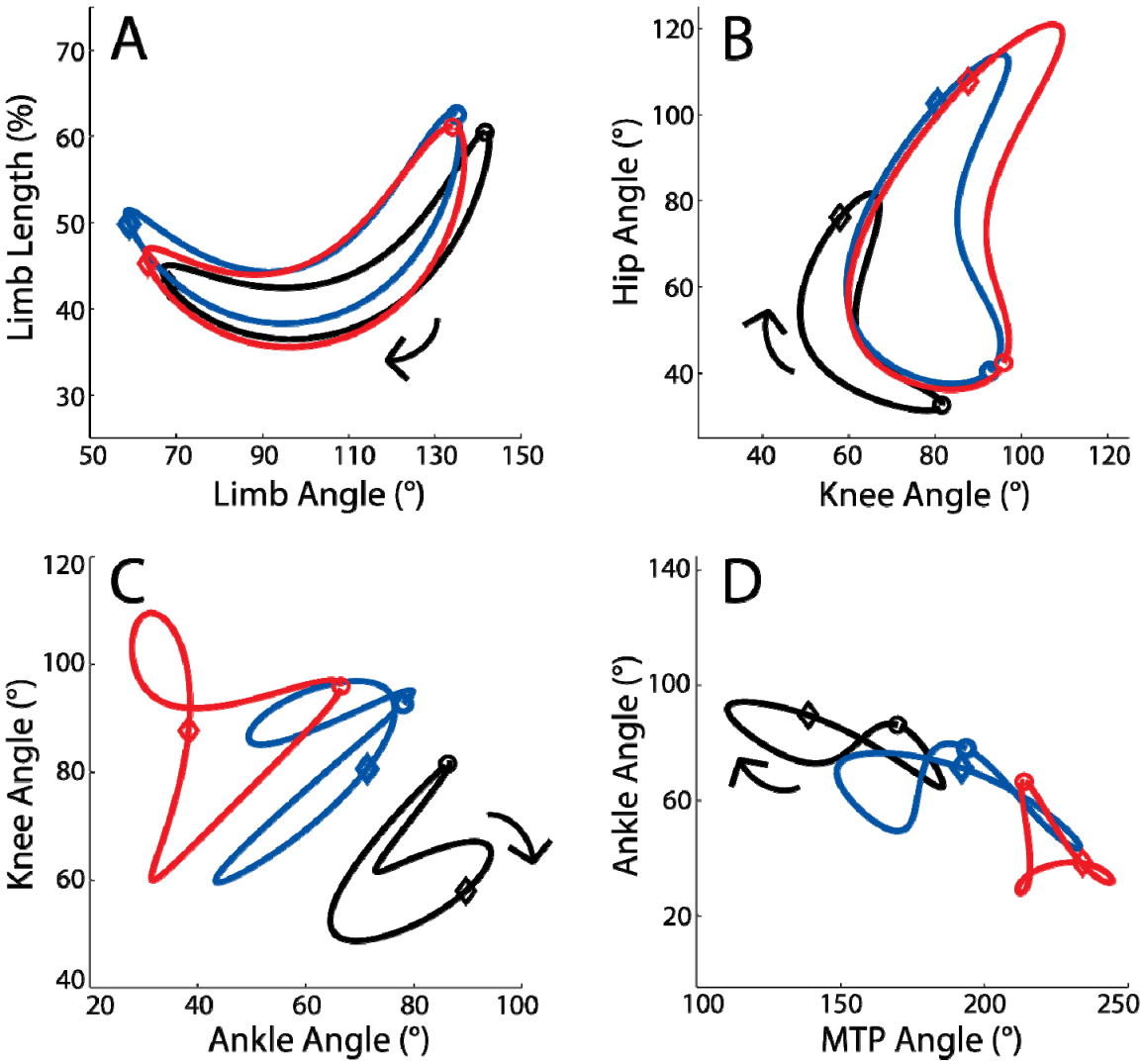

Figure 3-4. Coordination among joint and limb kinematic parameters
Coordination between limb length and angle (A) is represented by plotting each on their own axis. Analogously, angle-angle plots depict intralimb coordination between contiguous joint pairs: hip-knee (B), knee-ankle (C), and ankle-MTP (D). Stance phase begins with paw contact (circles) and swing phase with paw liftoff (diamonds). The direction of the gait cycle is counter-clockwise. These results share the same color conventions as previous figures.

Coordination among kinematic behaviors may be represented by plotting two joint angle trajectories against each other with each on their own axis (Fig. 3-4). Plotting the two limb kinematic parameters against each other (Fig. 3-4A) demonstrated that the pre-injury limb coordination relationship had been largely conserved. Yet coordination between pairs of contiguous hindlimb joints were all disrupted from the pre-injury condition. The hip-knee joint angle relationship (Fig. 3-4B) for both injury groups has roughly the same shape but very different positions in the state-space, mostly due to increased hip range of motion post-injury. The knee-ankle (Fig. 3-4C) and ankle-MTP (Fig. 3-4D) joint angle relationships were even more radically changed. The post-injury hip-knee trajectory was shared between severe and moderately injured rats, while the knee-ankle and ankle-MTP plots for moderately injured rats were more similar to control data than those of severely injured rats.

3.4 Discussion

Surgical denervation of the triceps surae muscles is a major constraint on ankle joint function during locomotion that significantly altered joint kinematics of the injured hindlimb. Both experimental groups exhibited yielding or increased flexion at the ankle joint during early stance phase. Importantly, this was compensated by just enough increased extension at the hip, knee, and MTP joints to maintain stable limb kinematics during post-injury locomotion. This general compensatory strategy persisted across severity of injury and different intralimb coordination patterns. This implies maintenance of normal limb kinematics, but not joint kinematics, is a goal of injury compensation during legged locomotion. We thereby accept our hypothesis the pre-injury limb kinematics would be conserved.

Mean coefficients of determination for the moderate injury group at each joint were significantly greater than their severe group counterparts (Table 3-1). Though only one joint was targeted by surgical intervention, each hindlimb joint exhibited changes in

its kinematics pattern. We thereby accept our hypothesis the pre-injury joint kinematics of each joint would be disrupted.

Rats maintained pre-injury kinematics whether or not the medial gastrocnemius muscle was intact. Sparing medial gastrocnemius predictably resulted in less post-injury ankle flexion than when all four muscles were denervated. Yet when one major ankle plantarflexor was left intact, there were less dramatic changes in individual knee, ankle, and MTP joint angles as well. In cats, this injury model produces increased EMG activity of the remaining muscle (Pearson et al. 1999). This increased activation could explain why the ankle is less flexed in moderately injured than severely injured rats. As to differences within the trend of increased extension of the hip, knee, and MTP joints, this could be explained through changes in inhibitory autogenic feedback between the ankle plantarflexors and other muscle groups (Ross and Nichols, 2009). Denervating four muscles may reduce inhibition throughout the limb to a greater extent than denervating three muscles, culminating in greater muscle activity and ultimately extension at the other joints.

We represented all kinematics in terms of a gait cycle normalized in time. While the peripheral nerve injuries tended to reduce stance duration of the injured limb relative to controls, the results were not statistically significant across all animals. Moreover, we wanted to treat the limb kinematics trajectories as a task-level for the entirety of locomotor behavior, not merely when that limb is in contact with the ground. That mean limb kinematics over the entire stride cycle are conserved despite potential changes in relative stance and swing duration reinforces the importance of the entire limb kinematic trajectory to the control of locomotion.

Both of our experimental models represent long-term compensation strategies to peripheral nerve injury. After verifying that post-injury changes in kinematics had stabilized after one week, we pooled all data for each injury condition. The emphasis on long-term compensation was reinforced through our choice of denervation as opposed to

self-reinnervation. Self-reinnervation, often performed in cats, only temporarily removes motor function while permanently removing some aspects of sensory feedback, such as the monosynaptic stretch reflex (Abelew et al. 2000; Maas et al. 2007; Chang et al. 2009). The recovery of muscle function during the paralytic stage after self-reinnervation could change the final post-injury kinematics trajectories. Our study removes any such variability as motor and sensory function do not recover after permanent denervation. Thus, the observed behaviors are more consistent representations of the long-term compensation strategies.

Every instantaneous limb length and angle adopted during locomotion may be achieved through an infinite combination of goal-equivalent joint angle configurations. During human hopping, joint kinematics differ with each hop, but act together to stabilize limb kinematics over many cycles (Ivanenko et al. 2007; Chang et al. 2008; Auyang et al. 2009; Yen et al. 2009; Yen et al. 2010). If the mechanism of limb function preservation by intralimb coordination is a fundamental principle of motor control, then temporary deviations may be treated in the same manner as chronic physiological deficits. This has been shown to be the case for cats that have experienced peripheral nerve injury (Chang et al. 2009); we suggest here that it is also the case in rats with peripheral nerve injury. These findings also represent a third mammalian order to exhibit this phenomenon for locomotor compensation, which suggests this trait may be conserved for terrestrial locomotion.

These results along with recent neurophysiologic experiments implicate a critical role for limb kinematics within motor control. The central nervous system is suggested to integrate limb postural information within the spinal cord into representation of whole limb behavior (Bosco and Poppele, 2000; Bosco et al. 2000; Poppele et al. 2002; Bosco et al. 2006). The central nervous system may also issue motor commands that correspond to these same neural representation of limb kinematics, which could be decoded at the spinal level to coordinate the available motor redundancy among joints. This line of

reasoning agrees with recent findings that the execution of descending motor commands involves the activation of combinations of synergistic motor units because both approaches posit low dimensional control variables being represented in the spinal cord (Ting and Macpherson 2005; Tresch et al. 2006; Ting and McKay 2007; Clark et al. 2010).

There are other physiological mechanisms that could be involved in stabilizing limb kinematics after injury. The gastrocnemius muscle exerts heterogenic inhibitory force feedback on biarticular muscles resulting in reinforced coordination between the knee and ankle joints (Ross and Nichols 2009). This could factor into how well the moderate injury group matches controls, but does not explain the performance of the severe injury group as both heads of the gastrocnemius were denervated for those rats. The emergent joint kinematics may also be a product of passive biomechanical mechanisms as well, such as the anatomical connections of biarticular muscles and interaction torques between limb segments. However, further study would be needed to quantify the relative contributions of passive mechanics vs. neural control.

That limb kinematics are conserved while joint kinematics vary from their pre-injury configuration suggests that limb kinematic trajectories are more critical to locomotion than those of the constituent joints. The mechanism by which animals achieve this compensation strategy is likely a combination of adaptation to different post-injury afferent sensory feedback signals as well as changes in descending motor commands. If maintaining any particular pre-injury hip, knee, or MTP trajectory were of a higher priority to locomotor control than that of the limb, then our injury models would not prevent that compensation strategy from occurring. That this does not occur reinforces the notion that the joints are not independent actuators but rather the product of integrated muscle and limb segment actions that serve a greater goal. We expect this compensation strategy to extend to all nerve injuries affecting a single joint; should

multiple joints become compromised, it is possible that the task of preserving limb kinematics would be sacrificed to maintain locomotion despite extensive injury.

3.5 Conclusions

Anatomical redundancies of the neuromuscular system promote the beneficial property that failure of one part does not impede the function of the whole. Conservation of limb kinematics is a task goal of locomotion that persists despite permanent disruption of individual joint kinematics. The manipulation of motor redundancies at one anatomical level to satisfy the goals of a higher level may be a universal compensation mechanism for motor control.

CHAPTER 4

CONSERVATION OF BILATERAL LIMB FUNCTION AFTER PERIPHERAL NERVE INJURY

4.1 Introduction

The body's response to injury often involves changes to the behavior of physiological structures or systems apart from those immediately affected. Peripheral nerve injuries to cat and rat ankle extensor muscles induce changes among hindlimb joint kinematic trajectories during locomotion that conserve pre-injury limb kinematics (Chang et al. 2009; Chapter 3). Yet the extent of this coordinated relationship between joint and limb function is unexplored. It is unknown if a strategy similar to that of intralimb coordination exists among other anatomical levels after injury, such as muscles coordinating to stabilize joint function, or limbs coordinating to stabilize the center of mass. We addressed the question of what interlimb property of locomotion the individual limbs may be coordinated to stabilize.

Studies of pathological gait have often focused on characterizing the deviations from symmetry of normal gait. This may be based on the assumption that symmetric gait is more energetically efficient than asymmetric gait (Donker and Beek, 2002). While traditional measures of gait asymmetry were often limited to comparing measurements derived from a single hindlimb (e.g. normalized difference of stance durations or step lengths), a proper and full account should be based on a bilateral comparison of the entire gait cycle (Sadeghi, 2003). One example of a more rigorous evaluation of motor symmetry is dynamical systems theory, which quantifies symmetry in terms of the relative phase between coupled oscillators (Jeka and Kelso, 1995). Dynamical systems

theory posits that coupled oscillators are more stable when they have identical properties rather than asymmetric patterns (Sternad et al. 1996). We will test the general hypothesis that after injury the limbs are coordinated in a way that preserves pre-injury bilateral symmetry of gait.

After unilateral injury, interlimb coordination of locomotion is typically characterized by gait asymmetry, or more commonly "limping." Colloquially limping may be characterized visually, but the term most often describes antalgic gait, which is defined by asymmetry in stance and swing durations between limbs (DeVisser et al. 2005; Sawyer and Kapoor, 2009). Gait asymmetry typically manifests as differences in step length ratio and relative limb phase (Donker and Beek, 2002; Hsu et. al., 2003; Balasubramanian et al. 2007; Balasubramanian et al. 2008;). Gait asymmetry after unilateral injury of varying severity in rats also occurs as asymmetries of vertical force and positive work exerted between the hindlimbs (Muir and Whishaw, 1999; Webb and Muir, 2003; Webb and Muir, 2004; Bennett et al. 2012). In these instances, the uninjured limb typically increases its relative stance duration or force output in proportion to decrements in those of the injured limb. Bilateral compensation may be a fundamental aspect to limping after unilateral injury.

In addition to a lack of motor output, limping may be the product of important changes in afferent sensory feedback due to injury. Control of the transition between extension and flexion phases of locomotion is typically modeled by the mutual inhibition of flexor and extensor half-centers of a central pattern generator; the mutual inhibition itself is regulated through a combination of bilateral feedback sources (Duysens, 2000; Rossignol, 2006; Pearson, 2007). Initiation of the flexor phase of the locomotor rhythm requires the integration of feedback from (*1*) hip extension, as measured by muscle spindles in the hip flexor muscles, (*2*) reduced load on the ankle joint, and (*3*) increased load across the contralateral ankle joint, as perceived by Golgi tendon organs in the ankle extensors (Dietz and Duysens, 2000; Pearson, 2007). Injury can change sensory

conditions directly, through damage to afferent nerve fibers, or indirectly, by way of compensatory behaviors. Experimentally prolonging or reducing load-related feedback will respectively extend or shorten stance duration of that limb (Hiebert et al. 1995; Whelan et al. 1995a; Whelan et al. 1995b; Hiebert et al. 1999; Duysens et al. 2000; Hayes et al. 2012). The degree of gait asymmetry can then be thought of as resulting from the product of instantaneous differences in afferent sensory feedback between limbs.

When changes in bilateral limb load are superimposed over unilateral loss of motor and sensory function to ankle extensor muscles, we can expect the degree of gait asymmetry to change with respect to surface grade. For example, the external loading conditions experienced by both limbs can be manipulated by walking up- or downslope. Variation in surface grade increases or decreases the positive work required of the hindlimbs to maintain locomotion at a constant speed. Consequently, limb vertical ground reaction forces and ankle moments in quadrupeds changed proportionately with slope (Gregor et al. 2006; Lammers et al. 2006; Maas et al. 2009 After unilateral injury, the injured limb will often have reduced sensory function compared to the intact limb, which results in different afferent sensory feedback signals relayed back from each limb to the central nervous system. These differences are potentially magnified as the strengths of force- and length-dependent afferent feedback are increased with upslope and downslope conditions respectively (Abelew et al. 2000; Gregor et al. 2006; Maas et al. 2010). Limping may therefore be a response to environmental factors that manipulate afferent sensory feedback conditions.

The purpose of this study was to test whether rats with unilateral injury would preserve bilateral symmetry of gait. We previously determined that pre-injury limb kinematics were conserved after ankle muscle denervations due to compensation by the other hindlimb joints. If interlimb coordination operates to preserve bilateral limb symmetry, then since injured limb kinematics were unchanged, we would not expect a

compensatory response from the uninjured hindlimb. We first hypothesized that pre-injury bilateral symmetry of limb kinematics would be conserved.

As the severity of unilateral injury increases, greater responsibility for meeting the propulsive and gravity resisting demands of locomotion will fall on the contralateral or intact hindlimb. We varied the surface grade of locomotion such that interlimb coordination would elicit compensatory reactions by one or both hindlimbs. If bilateral symmetry is a goal of interlimb coordination, we would expect any compensation by the intact hindlimb to mirror those of the injured hindlimb and thereby preserve gait symmetry. However, at increased workloads with upslope locomotion, we would expect the motor and sensory deficits of the injured limb to become more pronounced, resulting in gait asymmetry. Conversely, as the limb load demands of locomotion decrease when travelling downslope, we would expect lesser manifestation of gait deficits and hence less gait asymmetry. We further hypothesized that bilateral symmetry of rat limb kinematics would vary in inverse proportion to the degree of surface grade and injury.

4.2 Methods

Eleven adult male Sprague-Dawley rats (Rattus norvegicus) were housed in pairs and provided food and water ad libitum. Each rat was acclimated to treadmill locomotion prior to testing. During data collection, the animals were enclosed within a Plexiglas treadmill (Columbia Instruments) oriented perpendicular to the beam of a custom, high-speed X-ray video system (Bauman and Chang, 2010). A single trial consisted of 12 seconds of X-ray exposure filmed at 200 Hz. A data collection session yielded 10-12 trials per rat consisting of ~50 step cycles.

Each rat participated in a repeated measures study in which they locomoted over different surface grade conditions before and after an injury intervention. Rats were trained to walk at each slope condition prior to data collection. The treadmill was

mounted on a custom rig that could be set at five different slopes: -30°, -15°, 0°, +15°, and +30°. Treadmill speed was maintained at 0.4 meters/second for all conditions.

After data were collected for each rat and slope condition, peripheral nerve injuries were induced under isoflurane gas anesthesia and aseptic surgical conditions. Incisions were made in the posterior popliteal fossa of each left hindlimb and branches of the tibial nerve leading to the medial and lateral gastrocnemius, soleus, and plantaris muscles were exposed. One of two surgeries were performed on each rat: a "severe" denervation injury that permanently paralyzed all four major ankle plantarflexor muscles, or a "moderate" injury that denervated only lateral gastrocnemius, soleus, and plantaris but left medial gastrocnemius intact. Nerve branches were identified visually with blunt dissection and then verified with electrical stimulation. The nerve branches were then transected at their most proximal and distal branch points and an approximate 5-10 mm section was removed. Denervation of target muscles was verified by electrical stimulation of the proximal portion of the tibial nerve before wound closure. Muscles were stimulated again during a terminal surgery to further confirm denervation as well as to verify the absence of reinnervation. We performed all procedures in accordance with a protocol approved by the Georgia Institute of Technology IACUC.

After at least seven days post-injury, rats again participated in kinematic data collection after receiving the severe (n=5) or moderate (n=6) surgeries. Kinematics were measured anywhere from one to four weeks post-injury and the data was pooled across all time points. Experimental conditions maintained the same treadmill speed and surface grades as pre-injury testing. One rat from each surgery group refused to walk at the most extreme locomotor conditions (-30°, +30°).

Joint and limb kinematic data were extracted from sagittal plane X-ray videos. Video data were first corrected for image distortion and enhanced for contrast (NI Vision, National Instruments; Bauman and Chang 2010). Hindlimb features were manually digitized frame-by-frame using open source MATLAB software (Hedrick, 2008). Marker

position data were low-pass filtered (7 Hz) and used to derive limb and joint kinematics as depicted in Figure 4-1. Six anatomical landmarks (4th distal phalanx, 4th metatarsal head, lateral malleolus of the fibula, lateral epicondyle of the femur, greater trochanter, and the caudal margin of the ischium) defined four included joint angles (metatarsophalangeal (MTP), ankle, knee, and hip joints) on the injured hindlimb. The limb kinematic vector of each hindlimb was calculated from the 4th distal phalanx marker to that of the greater trochanter. Limb length and angle were measured for both hindlimbs. Limb length was normalized to the sum of the lengths of the four injured hindlimb segments.

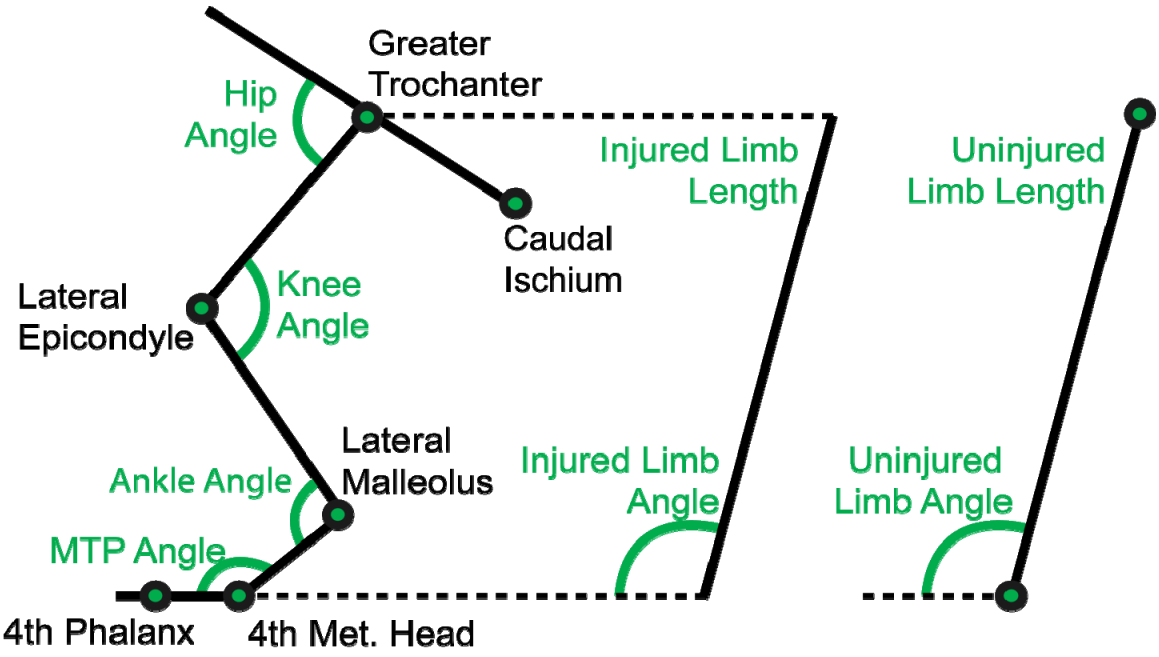

Figure 4-1. Rat kinematic model extended to include the uninjured hindlimb
Anatomical landmarks (black text) and sagittal plane joint and limb kinematic variables (green text) are depicted during stance phase. Two markers defining the uninjured limb vector were recorded in addition to six of the injured hindlimb.

Each stride was time-normalized to one hundred percent of the gait cycle. Mean kinematics trajectories were calculated for each animal, and then averaged across

individuals to report grand mean trajectories for each experimental condition. We used linear regression to statistically compare kinematics bilaterally. We calculated coefficients of determination (R^2) between each injured and uninjured limb trajectory. We then performed a t-test of the hypothesis that the mean R^2 value across rats within each surgery group would explain the majority of the variance of bilateral symmetry parameters of the control rats, i.e. mean R^2 will be significantly greater than 0.5. We used this 0.5 value as a threshold for determining symmetry of kinematics. The regression and t-test calculations were performed with MATLAB software.

We also measured other gait parameters to interpret our limb kinematics results, including normalized stance duration (i.e. duty factor), double support times, and the relative timing of joint angle extreme values. Stride parameters such as duty factor and double support were calculated from visual inspection of the paw contact and liftoff events of both hindlimbs. Duty factor was defined as stance phase duration normalized to stride duration. Double support times are the periods when both limbs are on the ground; there are two different double support times in each gait cycle.

4.3 Results

4.3.1 Bilateral limb symmetry after peripheral nerve injury

During level walking, bilateral symmetry of limb angles was conserved after unilateral denervation of ankle plantarflexors. The mean R^2 values comparing limb angles bilaterally were significantly greater than the 0.5 threshold among severe (0.780 ± 0.206) and moderately injured rats (0.937 ± 0.062) for level walking (Figs. 4-2 A, B). However, bilateral limb length symmetry was not conserved with either surgical intervention. Coefficients of determination between limb length trajectories were not significantly greater than threshold (severe: 0.533 ± 0.129 and moderate: 0.712 ± 0.264) (Figs. 4-2 C,

D). The mean limb angle and limb length plots demonstrate the extent that changes to individual limb trajectories contributed to bilateral asymmetry.

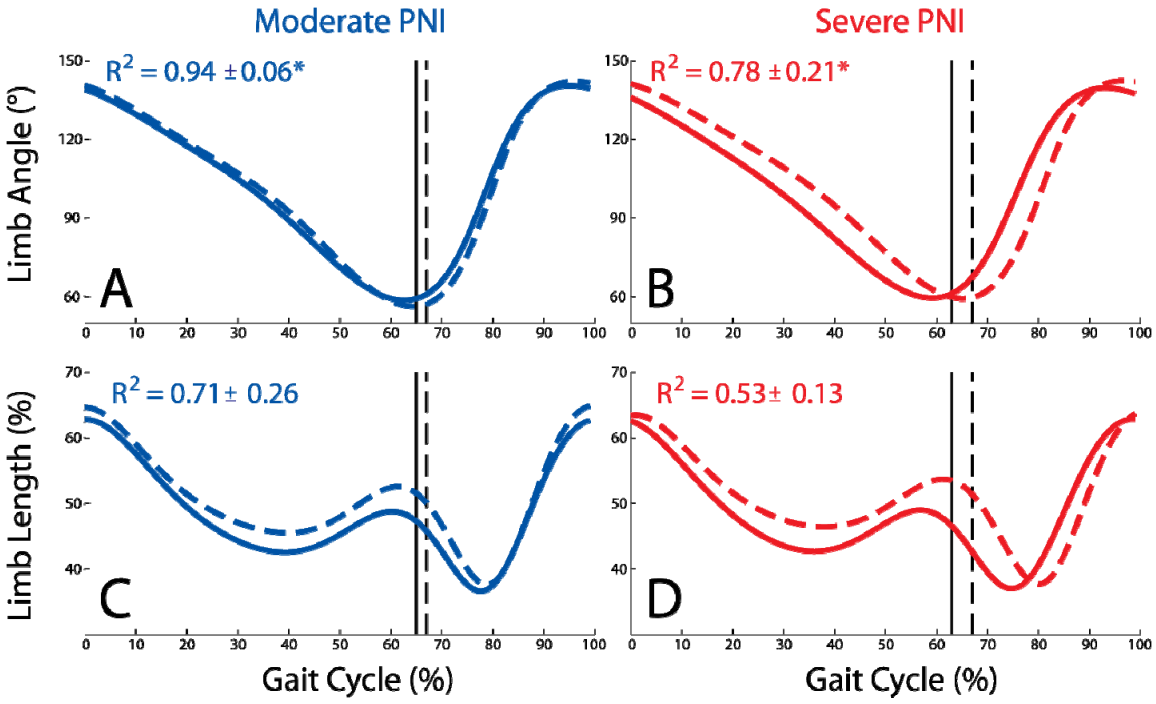

Figure 4-2. Bilateral symmetry of limb angle was conserved after peripheral nerve injury (A,B), but bilateral symmetry of limb length was not conserved (C,D). Mean injured (solid) and uninjured (dashed) hindlimb kinematics trajectories were plotted over the normalized gait cycle after complete (red, n=5) and partial (blue, n=6) triceps surae denervations. The vertical bars represent the transitions between the stance and swing phases of gait for the injured (solid) and uninjured (dashed) hindlimbs. Mean coefficients of determination (R^2) compared the trajectories bilaterally.

4.3.2 Bilateral limb angle symmetry versus slope

Bilateral symmetry of limb angle was conserved across all animals in nine of ten combinations of peripheral nerve injury and slope condition (Fig. 4-3). For moderately injured rats, the average R^2 value comparing injured and uninjured trajectories was significantly greater than 0.5 at all slopes. Rats that experienced the severe nerve injury

maintained limb angle symmetry at all slopes except -30° downslope condition, at which the hindlimb angles became highly dissimilar. We can quantify the trends by calculating

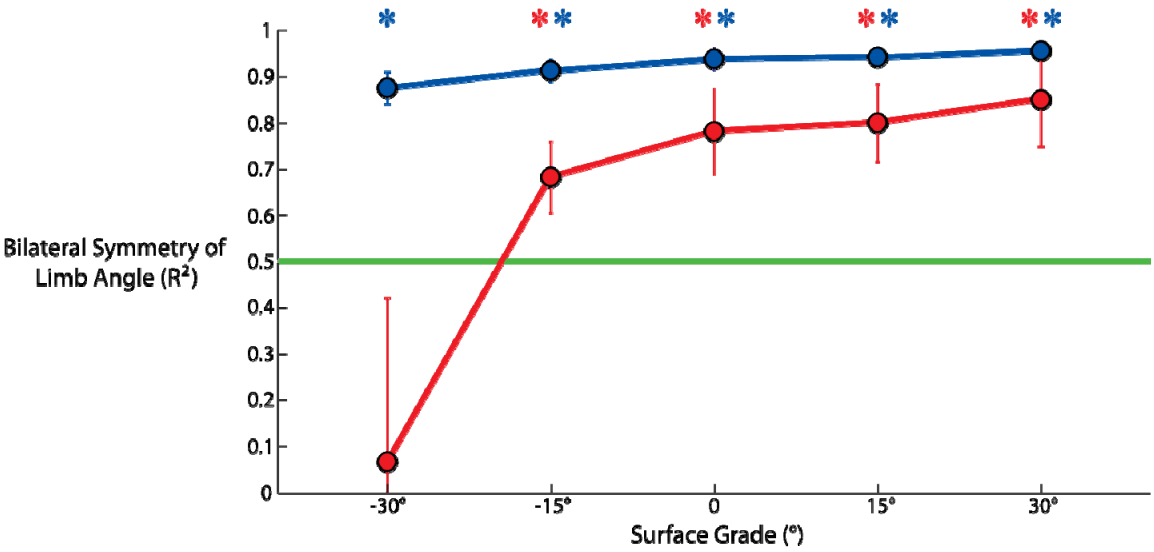

Figure 4-3. Bilateral symmetry of limb angle was largely conserved across surface grades.
Coefficients of determination (R^2) correlating post-injury limb angle trajectories of the injured and uninjured hindlimbs were calculated for five slope conditions and two surgical interventions: complete Triceps surae (red) and partial Triceps Surae (blue) denervations. R^2 values were then averaged across rats. $R^2 = 1$ signifies complete correspondence between data sets. $R^2 < 0$ signifies that the mean value of post-injury kinematics over the gait cycle is a better fit to the control data than the trajectory itself. Asterisks denote when post-injury kinematics explained greater than 50% of the variability of control data ($p<0.05$)

the slope of R^2 values across changes in surface grade for each rat and then performing a t-test on the mean slope coefficients. The degree of bilateral symmetry of limb angles (R^2) increased as surface grade increased after both injuries ($p<0.006$ for severely injured rats; $p<0.02$ for moderately injured rats).

For severely injured rats, this pattern of bilateral limb angle bilateral symmetry decreasing as slope decreased was consistent with trend in hindlimb duty factors (Fig. 4-4). The hindlimbs of severely injured rats exhibited significant asymmetries in duty factor in the downslope conditions. These asymmetries were due to significantly shorter stance

durations of the injured limb compared to its own pre-injury values; duty factor of the intact hindlimb did not change when walking downslope (Fig. 4-5). The moderate

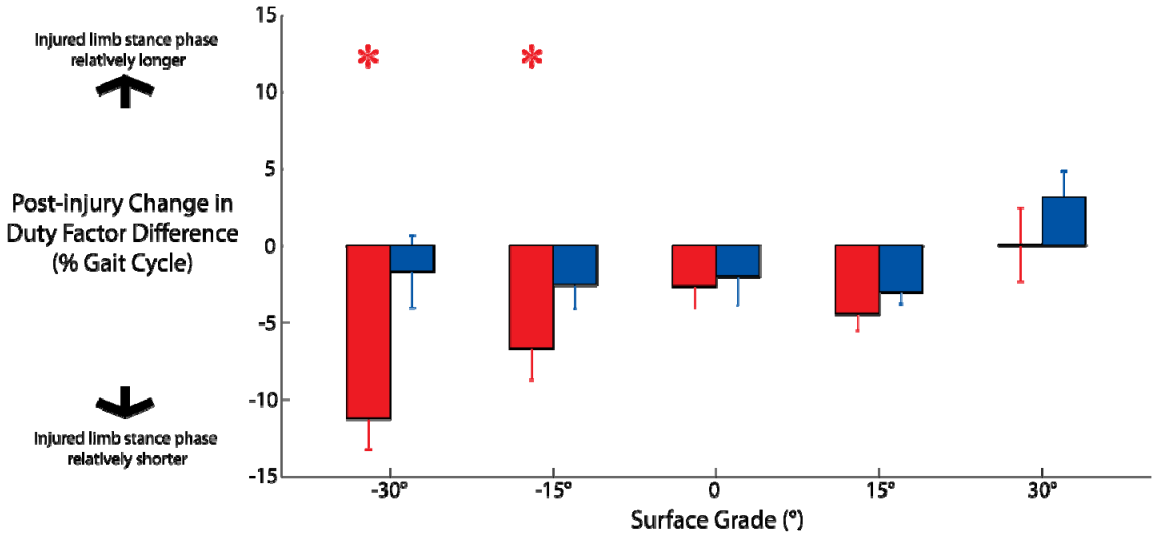

Figure 4-4. Bilateral asymmetry of duty factor increased as slope decreased for severely injured rats.
Post-injury changes to bilateral difference in normalized stance duration were calculated for all five slope conditions and both moderate (blue) and severe (red) injuries. Asterisks denote statistically significant differences from zero ($p<0.05$).

surgery on the other hand induced no significant changes in bilateral symmetry of duty factor.

Changes in peak hip extension timing of rats with the severe ankle plantarflexor injury corresponded to earlier transitions from stance to swing phase post-injury. The timing of peak hip extension occurred significantly earlier in severely injured rats at the -30°, -15°, 0°, and +15° slope conditions (Fig. 4-6). At the middle surface grades the timing changes were independent of the magnitude of hip extension; only in the -30° condition did the hip reduce its range of hip flexion post-injury. As was the case for duty factor, the absence of changes in peak hip extension timing for moderately injured rats was consistent with preservation of bilateral limb angle symmetry at all slopes.

4.3.3 Bilateral limb length symmetry versus slope

Bilateral symmetry of limb length was tended to decrease as surface grade increased (Fig. 4-7). However, the slopes of the R^2 values versus grade was not significantly different than zero. The average R^2 value for moderately injured rats was

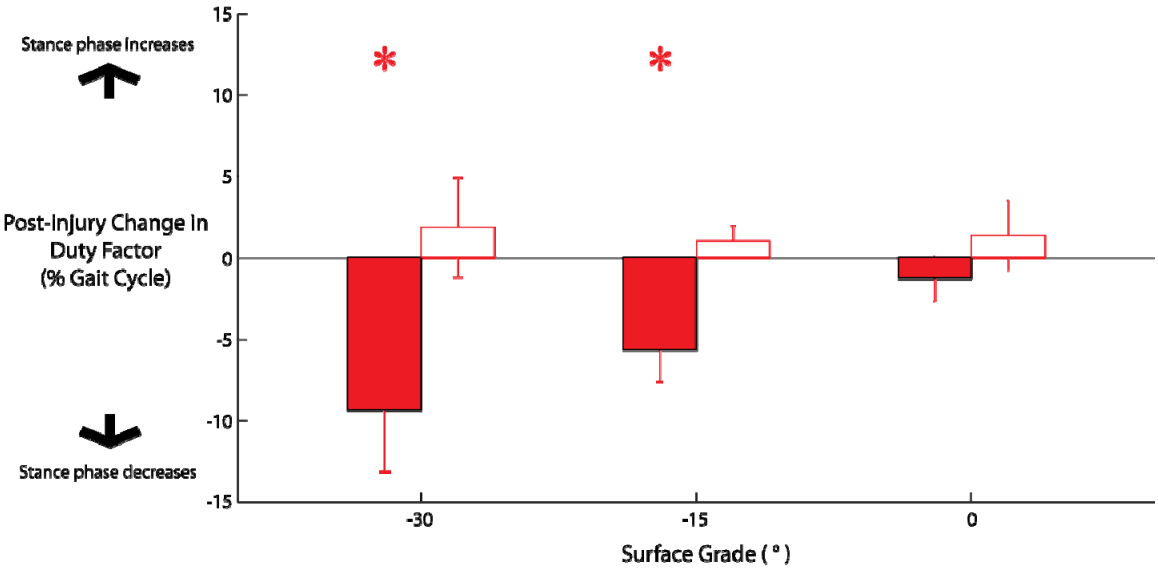

Figure 4-5. Bilateral asymmetry of duty factor in severely injured rats was due solely to reduced stance duration of the injured hindlimb.
Post-injury changes to normalized stance duration of the injured (solid) and uninjured (empty) severe rat hindlimbs were calculated at the level and downslope conditions. Asterisks denote significant differences from zero (signifying pre-injury values) ($p<0.05$).

significantly greater than 0.5 at only the -15° and -30° slope conditions, whereas severely injured rats only conserved limb length bilateral symmetry at -30°. Both groups exhibited limb length asymmetries within the upslope treadmill conditions. Neither injury group exhibited coefficients of determination greater than threshold in level or upslope walking, indicative of bilateral limb length asymmetry in these conditions.

Disruption of bilateral limb length symmetry in severely injured rats was representative of changes in limb length around the paw off event of the gait cycle (Fig. 4-8). This group demonstrated significant bilateral differences in limb length at paw off for the -15°, 0°, +15°, and +30° slopes. Asymmetries in limb length for severely injured rats, at least a

six percent difference on average, matched the slope conditions at which they did not conserve bilateral symmetry of limb length.

Asymmetries in bilateral limb length for moderately injured rats arose from more subtle kinematic changes. Rats who received the moderate surgery also exhibited statistically significant differences in limb length at paw off across all but one surface grade (Fig. 4-8). However, these values were smaller than those of severely injured rats and did not correlate with the pattern of limb length asymmetry while walking upslope. Limb length asymmetry in the moderate group was better represented by changes in duration of one of the two double support phases of each stride cycle (Fig. 4-9). As slope increased, moderately injured rats significantly increased the proportion of the double support phase in which weight was transferred from the injured limb to the uninjured limb.

Bilateral symmetry of limb kinematics was better conserved by moderate than severely injured rats. Mean coefficients of determination for limb angle and length symmetries were never smaller than those of moderately injured rats at any slope condition. Yet, both injury interventions shared the trends of increased limb angle symmetry and decreased limb length symmetry in relation to increases in surface grade.

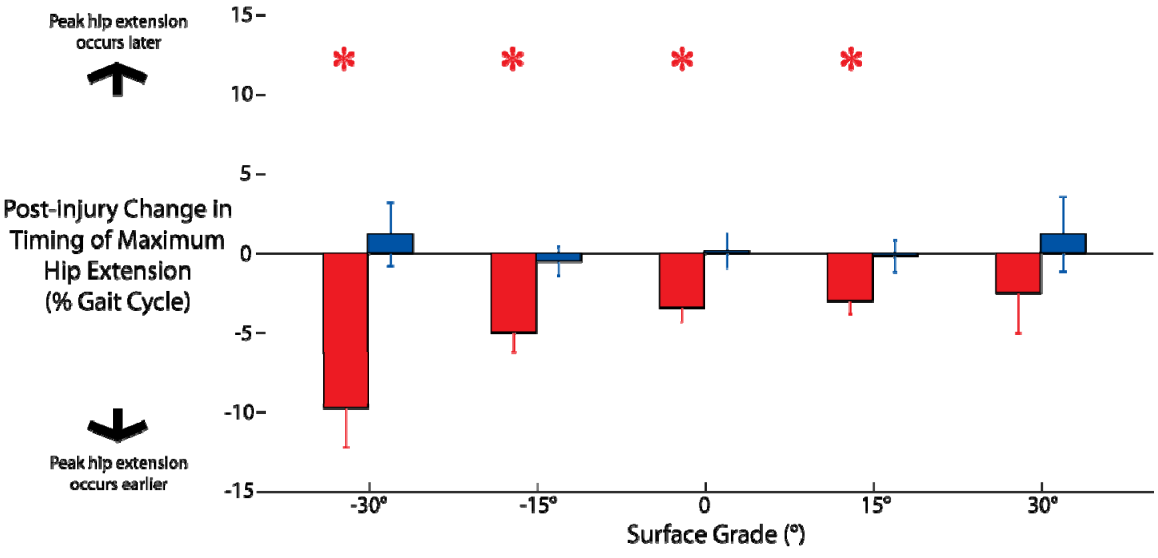

Figure 4-6. Timing of maximum hip extension occurred relatively earlier as slope decreased for severely injured rats.

Post-injury differences in the percentage of stride duration at which hip joint angle of the injured hindlimb is maximized were calculated for all five slope conditions and both moderate (blue) and severe (red) injuries. Asterisks denote significant differences from pre-injury values ($p<0.05$).

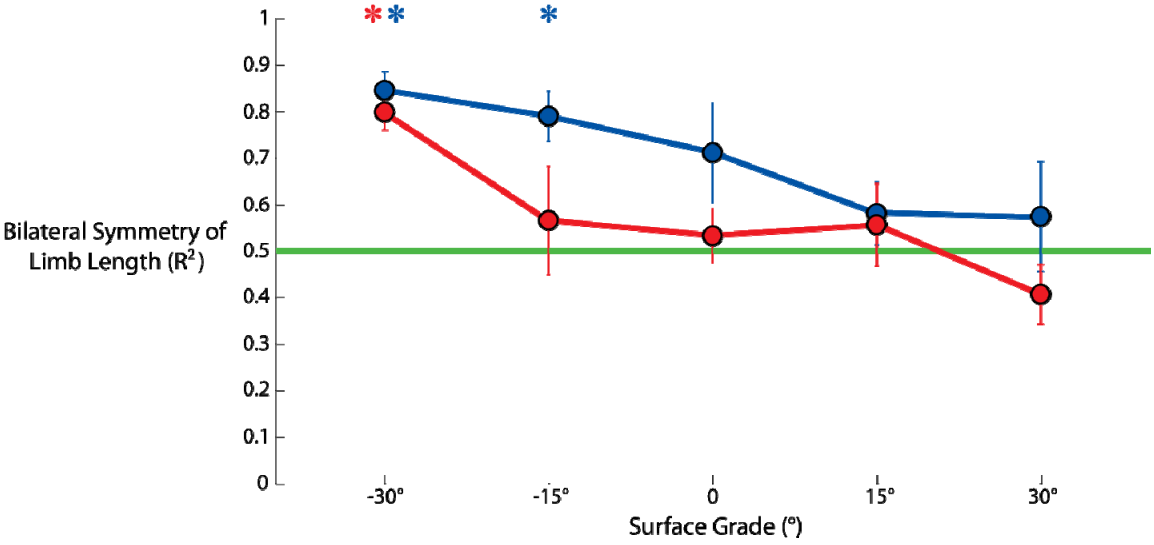

Figure 4-7. Post-injury bilateral symmetry of limb length was only present in downslope walking.

Average coefficients of determination (R^2) relating post-injury limb length trajectories of the injured and uninjured hindlimbs were calculated for all five slope conditions and both moderate (blue) and severe (red) injuries. The data follow the conventions of Figure 4-3.

4.4 Discussion

We hoped to determine whether preservation of bilateral limb symmetry was a principle of compensation to injury to rat ankle extensor muscles. Conservation of pre-injury limb kinematics after peripheral nerve injury was confirmed for multiple taxa and combinations of denervated muscles (Chang et al. 2009; Chapter 3). If interlimb coordination operated on similar principles to intralimb coordination, then since injured limb kinematics were unchanged we would not expect a compensatory response from the intact hindlimb. Our initial hypothesis was that bilateral symmetry of limb kinematics would be conserved in level walking. Average coefficients of determination for bilateral

limb angles were significantly greater than 0.5 after both injury interventions, but R^2) values for bilateral limb length were not significant. We accept this hypothesis with respect to limb angle, but reject it for limb length. Rejection of these hypotheses does not necessarily mean that interlimb coordination is of a lesser priority or lower in the hierarchical organization than intralimb coordination, the goal of which is conserved. The results could mean that despite preservation of injured limb kinematics, the uninjured hindlimb may compensate for deficits in some other aspect of injured limb function, for example limb extension force.

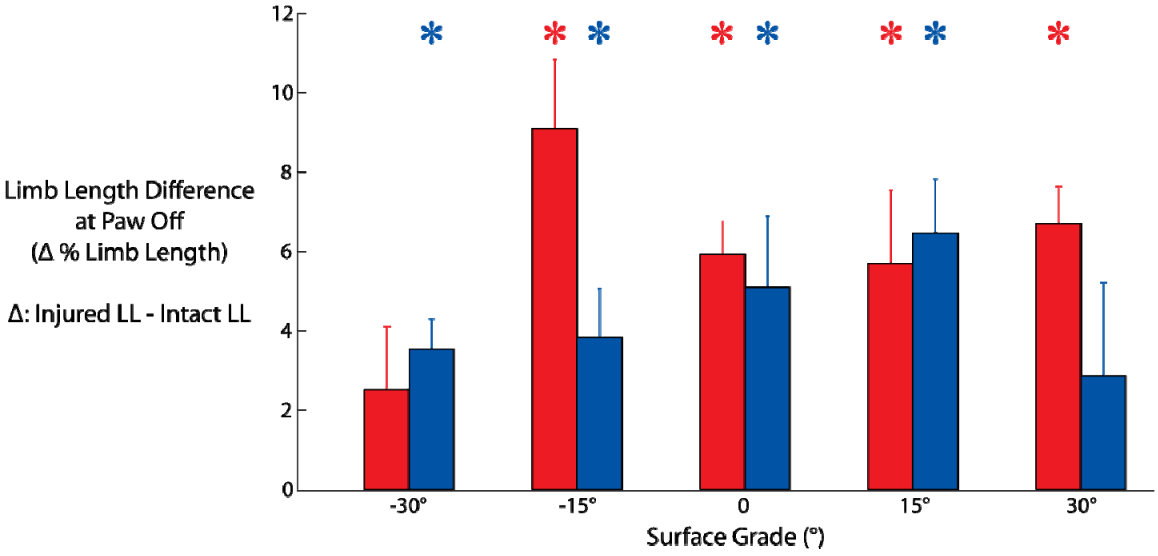

Figure 4-8. The intact limb is longer than the injured limb at paw off after PNI. Post-injury changes to bilateral difference in limb length at paw off were measured for all five slope conditions and both moderate (blue) and severe (red) injuries. The bilateral difference is calculated as "intact limb length – intact limb length" such that positive values indicate the intact limb is longer than the injured limb at its respective moment of paw off at the end of stance phase. Asterisks denote statistically significant differences from zero ($p<0.05$).

Bilateral symmetry of limb angle was largely conserved across variation in surface grade, as it was significantly greater than threshold in nine of ten conditions. Within this super-threshold region, the magnitude of limb angle symmetry subtly

decreased as surface grade decreased. We reject our hypothesis that bilateral symmetry of limb angles would vary inversely with increasing slope. These changes can be explained by an interlimb coordination pattern that reduced stance duration of the injured hindlimb while walking downslope. The relative timing of peak hip joint angle extension may be interpreted as representative of the afferent feedback signal from hip muscles that helps initiate transition to swing phase. Observed earlier peak hip extension post-injury was therefore consistent with smaller duty factors on the injured side. These results evince a general injury compensation strategy in which rats with severe peripheral nerve injuries respond to the reduced loading demands of downslope locomotion by reducing the relative work done by the injured hindlimb, whereas rats with moderate peripheral nerve injuries demonstrate no significant changes in the injured limb kinematics.

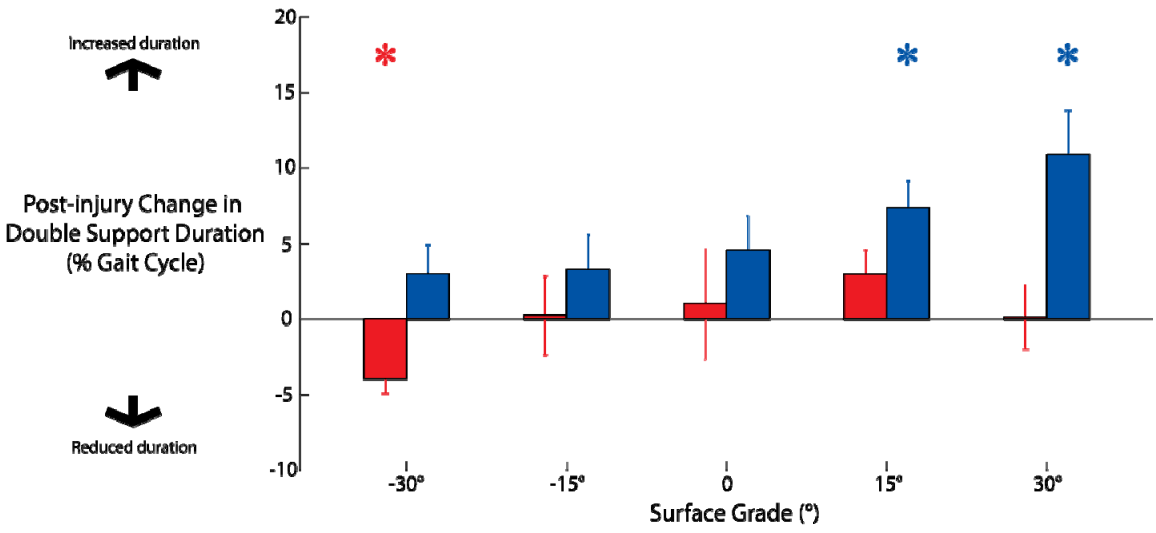

Figure 4-9. Moderately injured rats exhibited asymmetries in double support duration when walking upslope.
Post-injury changes to the normalized percentage of stride duration beginning with paw contact of the uninjured hindlimb and ending with paw off of the injured hindlimb were calculated from visual inspection of paw contact and liftoff events. Durations were measured for all five slope conditions and both moderate (blue) and severe (red) injuries. Asterisks denote significant differences from pre-injury values ($p<0.05$).

Bilateral symmetry of limb length was largely disrupted across variation in surface grade. Both surgery groups only demonstrated limb length symmetry within downslope conditions. Overall, bilateral symmetry decreased as surface grade increased. We accept our hypothesis that bilateral symmetry of limb length would vary inversely with slope. After peripheral nerve injury equivalent to the moderate surgery, rats generated less positive mechanical work with the injured hindlimb and greater positive work with the uninjured hindlimb compared to pre-surgery values (Bennett et al. 2012). For the severe ankle plantarflexor injury group, bilateral asymmetry of limb length can be explained entirely by this difference in work output. When walking upslope, severely injured rats likely responded to increased net loading demands of upslope locomotion by increasing the work done by the uninjured hindlimb in greater proportion to that of the injured limb. Despite expected asymmetries in kinetic measurements between moderate hindlimbs, this does not necessarily produce the same limb length asymmetries due to greater residual function of the injured ankle. For moderately injured rats, bilateral asymmetry of limb length correlated with longer post-injury durations of double support when weight is transferred from the injured to the uninjured limb. Significant increases to this double support phase could preserve the average impulse generated by the injured limb by exerting a reduced peak limb force over a greater period of time. This potential strategy, along with observations of a slight relative increase in uninjured limb length over late stance, is consistent with an interlimb coordination strategy comprising distinct compensations by each hindlimb with a relatively small disparity in positive work. Relatively greater loads on the contralateral limb with upslope walking could explain our results through reinforcing positive force feedback during contralateral stance phase (Duysens et al. 2000). Repetition of the experiments while collecting ground reaction forces would be necessary to confirm these conclusions.

Dissonance between compensation strategies governing these two representations of limb geometry reinforces the notion that limb kinematics may not be as fundamental to

interlimb coordination as they are to intralimb coordination. Rather, the significance of limb angle to interlimb coordination may be limited to the extent it is intrinsically tied to stretch of hip flexor muscles, a connection enhanced in downslope walking when length feedback predominates. Likewise, the physiological relevance of limb length may represent changes in limb kinetics, such that when the limb exerts a greater ground reaction force this corresponds with greater extension of the joints and a consequent increase in limb length. This conclusion is consistent with neural recordings that suggest that limb angle and limb force are encoded in the spinal cord through the integration of afferent feedback information (Bosco et al. 2006). Humans too appear to control leg angle and leg force during hopping, running, and walking gaits. (Yen and Chang, 2010; Yen, 2011; Toney and Chang, 2012). It is possible limb kinematics are emergent properties of locomotion related to another injury compensation strategy, such as preservation of ankle mechanical output (Prilutsky et al. 2011). Future studies along this line of investigation should examine intralimb and interlimb coordination of limb kinetics after peripheral nerve injury to rat hindlimbs.

4.5 Conclusions

Symmetry of limb angles may be a task goal after locomotion, but symmetry of limb length does not appear to be a goal. The more severe injury led to disparate compensatory behaviors by each hindlimb, whereas the less severe injury revealed a more nuanced strategy of both limbs working in concert. Post-injury changes in limb kinematics may be explained through afferent sensory regulation of the stance to swing transition. Although bilateral limb symmetry did not appear to be a conserved property of post-injury locomotion, the results hinted that some other parameter- perhaps bilateral kinetics- is maintained through interlimb coordination.

CHAPTER 5

CONCLUSIONS

Locomotion, like other motor behaviors, typically requires little conscious attention. Yet beneath the surface the neuromuscular system manages innumerable elements to achieve even the simplest behaviors. The exact details of how motor behavior is controlled remain unknown. Uncovering the control strategies could facilitate the study of movement disorders, as well as improve modeling of locomotion and robots engineered based on those models.

This dissertation work sought to uncover the principles governing the implicit goals of the neuromuscular system in the control of locomotion. While the true neural targets for control by the nervous system cannot be presently known, I used experimental design to identify whether certain parameters serve as intermediary performance goals essential to locomotor behavior. A review of the literature and my results support the view that the structure of motor control is hierarchical in nature, such that the problems of coordinating multiple degrees of freedom are solved across a series of levels. My general hypothesis was that locomotion is controlled through coordination of motor functions at a specific hierarchical level to maintain the implicit goals of a higher level. To test this hypothesis, I performed denervation experiments on rodents to reveal the critical components of locomotion retained through injury compensation. Rodents are ubiquitous in science, despite a lack of accurate kinematic data. Locomotion encompasses a stereotyped, repeatable, necessary, universal set of behaviors. Injury removes some degree of function from the system, providing the stimulus for change in behavior among the system's elements. My experiments incorporated many common ingredients from classic studies of motor control.

5.1 Key Findings

To address the general hypothesis I chose to first develop a rat model of locomotor injury. This required an advancement in kinematics measurement technology in order to evaluate the performance of rat injury models (Chapter 2). I used simultaneous measurement to directly compare our X-ray derived kinematics data against that collected with skin markers. The results revealed considerable differences in marker positions (Figure 2-5) and consequently joint kinematics (Figure 2-4) between methodologies. Given that X-ray bypasses the measurement errors due to skin movement artefacts, this is a validation that our more rigorous techniques are justified to accurately measure rodent limb and joint motion. I looked deeper into the effects of posture and speed to investigate the origin of skin movement error and prescribe conditions when traditional skin markers would yield accurate results. Increasing treadmill speed reduced skin movement errors over the range of speeds tested (Figures 2-6, 2-7), and knee marker positional error was minimized around the limb posture associated with paw off (Figure 2-8).

I then utilized the high-speed X-ray video technology to address questions of locomotor compensation that were previously inaccessible with rodent models. First I identified intralimb coordination behaviors after unilateral peripheral nerve injury (Chapter 3). I performed two types of ankle plantarflexor denervation experiments to act as a constraint on joint function and then predicted how this would impact behavior of the entire limb. I found that the hindlimb joints (Figure 3-2) were all coordinated in a fashion that preserved pre-injury limb kinematics (Figure 3-3). Limb kinematics trajectories were maintained across both injuries and over the entire gait cycle. Both groups of injured rats exhibited increased ankle flexion, while the other hindlimb joints compensated with the appropriate net amount of extension in each case to restore pre-injury limb length and angle trajectories. These findings suggest that preservation of pre-injury limb kinematics is a fundamental injury compensation strategy, and that limb kinematics may serve as implicit performance goals of motor control.

Next I asked the question whether this phenomenon in which joints are coordinated to preserve limb function was one element in a greater hierarchical structure of motor control. This raised the question of performance goals at other hierarchical levels, and whether redundancy between the limbs was similarly manipulated to achieve these goals. To test this, I proposed that the two hindlimbs were coordinated to preserve pre-injury limb symmetry (Chapter 4). Having established that limb kinematics of the injured limb were preserved, I reasoned that there would be no stimulus to trigger compensatory behaviors in the intact or contralateral limb. However, I found that limb angle symmetry was preserved but limb length symmetry had changed after both moderate and severe ankle plantarflexor denervations. The intact limb exhibited unilaterally increased limb length during late stance, a likely compensation to increase ground reaction forces in service of some higher level performance goal.

Finally I manipulated the degree of interlimb coordination between the hindlimbs by changing the treadmill gradient. To chart the boundaries of this bilateral compensation pattern, I designed an experiment that would externally manipulate the magnitude of interlimb coordination. Altering the externally imposed work load was expected to change the task goals of locomotion. Bilateral symmetry of limb angles was preserved in almost all cases, except for the combination of severe injury and extreme downhill surface grade (Figure 4-3). Bilateral limb length, on the other hand, increasingly diverged from symmetry as the surface grade of locomotion increased (Figure 4-7). My findings suggest the implicit performance goals of locomotion may change in response to variation of the environment. The results are also consistent with the notion that coordination of lower-order motor elements to meet performance goals of higher-order elements is a feature of the hierarchical organization of motor control.

5.2 Research Questions

How does injury compensation reveal implicit goals of motor control?

In order to understand the implicit goals of motor control, it is useful to define the behavior of living organisms with theoretical control systems. To this end we address animal locomotion as a dynamical system. Dynamical systems theory argues that individual elements may be coordinated to accomplish specific motor tasks (Saltzman and Kelso, 1987). Under this theory, performance of individual elements will vary to compensate for fluctuations of related elements such that performance of the task itself is undisturbed. Across many repetitions of a behavior, or in the case of locomotion many gait cycles, variables essential to overall task performance are stabilized. Our approach is to investigate the consistency of physiological elements in the face of imposed variation in order to gain insight into the implicit goals that make up the overarching task goal of locomotion.

In many areas of scientific inquiry, a full understanding of function may not be achieved while the subject of study remains intact. At the atomic level, physicists have developed particle accelerators that reveal evidence of subatomic particles in the wake of controlled destructive collisions of high energy particles (Weidemann 1999). At the cellular level, protein complexes such as ion channels are removed from their native environments and examined *in vitro* to determine their chemical and electrophysiological reactive properties (Catterall 1988). At the organismal level, localized brain lesions have historically sparked the revelation of the cognitive and motor responsibilities of specific cortical regions. Two of the most famous discoveries are Broca's and Wernicke's areas, which are involved in the understanding and production of language (Purves et al. 2004). I adopted a similar philosophy with the use of injury compensation to study locomotion. By removing an element of the system, in this case by denervating specific ankle extensor muscles, I compromised one aspect of locomotion- ankle function- but allowed all other structures to compensate freely in response. This technique thereby revealed what was necessary and what extraneous to the expression of locomotion.

Injury compensation is an appropriate vehicle to address neuromuscular control because the induced changes involve a reorganization of the nervous system. This was demonstrated in a series of experiments in which cats underwent neurectomy of ankle flexors and spinalization, in alternating sequence (Carrier et al. 1997). One group of cats first received unilaterally denervation of the tibialis anterior and extensor digitorum longus, allowed to adapt and return to symmetrical walking, and then spinalized. The result was a highly disorganized and asymmetrical locomotor pattern, indicating that supraspinal regulation was involved in the maintenance of consistent walking. Another cat was first spinalized, allowed to heal, and then received the same denervation as the other group. This cat walked with a largely symmetrical gait, accounting for the expected loss of ankle flexion, demonstrating the capacity of the spinal cord alone to adapt to denervation. Ultimately both groups received the same treatments by the end of the experiment, but their gait deficits depended on the order of operations. Together these results suggest that injury compensation invokes plasticity of the nervous system at the spinal and supraspinal levels to maintain the capacity of locomotion. Hence, injury compensation may serve as an experimental perturbation to the control programs of locomotion.

Taken together, injury compensation is a useful tool to reveal the implicit goals of locomotion. First, I considered animal locomotion from the perspective of dynamical systems theory in the sense that the elements of the system coordinate over many cycles to stabilize some greater task goal. I then introduced an experimental perturbation into the system to look for coordinative changes among elements indicative of implicit goals necessary to task performance. Injury compensation was chosen as the perturbation because it induced changes not only among locally related elements, but also within the nervous system controlling those elements. This experimental model is appropriate and sufficient to identify the implicit goals of locomotion.

What are the implicit goals of locomotion?

Discussion of the implicit goals of locomotion straddles the line between theory and experiment. Any motor output stabilized over time may be considered a performance variable (Latash 2010). Both joint and limb kinematics from my experiments constitute performance variables. Yet we cannot directly ascertain whether these variables also serve as true targets of control, or whether the motor patterns of these particular elements constitute implicit goals actively attended by the neuromuscular system. While this may appear intractable, implicit goals may be conceptualized from a second perspective: utilizing hypothesized implicit goals as an experimental design tool. This consists of evaluating the persistence of performance goals in response to perturbations of a dynamical system. My approach is to examine potential implicit goals through the latter paradigm, and then deduce whether they satisfy the former definition as well.

In intact animals undergoing steady state locomotion, the limbs and joints exhibit regularity in their kinematic profiles across gait cycles. Yet to suggest that the nervous system is actively and constantly controlling all of these parameters simultaneously would be an unwieldy, unparsimonious control scheme. My experimental design constrained unilateral ankle function in rats but allowed the rest of the system to behave freely. The result was preservation of pre-injury limb length and angle trajectories of the affected limb at the expense of coordinated changes to pre-injury hip, knee, and MTP joint trajectories (Figures 3-2, 3-3). This suggests that maintenance of limb kinematic behavior may be an implicit goal of locomotion, but not so that of pre-injury joint kinematics.

Before we speculate on whether whole limb behavior is an actual neural target of locomotor control, we must first establish that there exists a representation of anatomical entities such as limbs and joints within the nervous system. In a series of experiments, Bosco and Poppele measured neuronal activity from a population of neurons in the cat dorsocerebellar tract (DSCT) in response to changes in hindlimb posture. They

discovered that these neurons encoded a kinematic representation of limb axis length and orientation (equivalent to my definitions within this dissertation) within the spinal cord (Bosco et al. 2000, Bosco and Poppele 2000, Poppele et al. 2002). When the cats were later tested during active stepping behavior, Bosco and Poppele reinterpreted the limb length encoding to potentially signify a representation of limb loading (Bosco et al. 2006). In contrast to evidence of limb function encoding in the central nervous system, there is a paucity of evidence that such locomotor representations exist for individual joints. Each of the major limb joints are spanned by multiple muscles, many of which are bi- and tri-articular. While animals can consciously actuate a single joint while leaving all others fixed, the neighboring joints are actively resisting the interaction torques impose by the inertia of the moving limb segments. Moreover, muscle synergies often involve groups of motor units such that their activation typically encompasses multiple joints (Ting and Macpherson 2005; Tresch et al. 2006; Ting and McKay 2007; Clark et al. 2010). None of this precludes the existence of individual joint representations in the nervous system, but it suggests their management would be more complicated than that of the limbs. However, even if these representations do not exist for joints it should not be taken that joints do not constitute a proper hierarchical level due to extent of their redundancy with respect to joint function and the redundancy of muscles that control the joints. Together these findings are consistent with my conclusions that individual joint representation is uncertain but that limb kinematics are a likely implicit goal of locomotor control.

A variety of physiological mechanisms could be involved in coordinating the joints to preserve limb function. Inhibition of the quadriceps by the triceps surae could facilitate knee extension when warranted (Wilmink and Nichols 2003). Heterogenic reflexes, particularly those from the triceps surae, could act to reinforce interjoint coordination in a way that preserves limb length and orientation (Ross and Nichols 2009). Decerebrate cats do not preserve limb length after denervation, confirming that

preservation of this task goal requires long-term adaptation by supraspinal networks of the nervous system (Stahl and Nichols 2011). An alternative explanation for preservation of limb kinematics after injury is that this is not the result of deliberate coordination by the nervous system but rather a byproduct of the mechanical design of the hindlimb. If the toe and hip joints of the hindlimb were considered to be fixed in space, then the surgically induced flexion of the ankle joint would be expected to cause compensatory extension at the hip and knee joints by purely passive mechanics. However the hip joint should not be considered to be at a fixed vertical position, particularly during single limb support with respect to the contralateral hindlimb. In this context, passive mechanics would dictate that ankle flexion should result in compensatory knee flexion due to the attachment of polyarticular gastrocnemius and plantaris muscles to the distal femur (thigh segment), even if those muscles are denervated. That this knee flexion does not occur during locomotion suggests purposeful control by the neuromuscular system to negate these passive effects. While passive mechanics undoubtedly play some role in post-injury joint coordination, they do not appear to be the driving force of observed compensation strategies.

Yet preservation of pre-injury limb kinematics cannot be considered an inviolable implicit goal, because after unilateral denervation the contralateral hindlimb exhibits significantly greater extension during late stance phase (Figure 4-2). These results could be reconciled in that the pre-injury kinematics trajectories of the intact hindlimb may be considered sufficiently preserved by the nervous system, or that strict adherence to the limb-level goal is sacrificed to meet some higher order implicit goal of locomotion. The deviations from pre-injury routine by the contralateral hindlimb in my experiments suggest that some class of parameter other than kinematics may serve as implicit goals of locomotion. Kinetic variables are a fruitful aim of investigation, as the application of force on the external environment is a fundamental aspect of locomotion. Humans hopping on one leg select joint torque combinations that stabilize the vertical ground

reaction force on the entire limb (Yen and Chang 2010). This seems analogous to my conclusions relating joint and limb kinematic function after injury. Net peak force was stabilized for one or both legs during human hopping (Yen 2011). During walking, net force was stabilized during the double support phase (Toney and Chang 2012). After moderate peripheral nerve injury in rats, the symmetrical pre-injury kinetic traces between hindlimbs shifted such that the injured limb generated less positive mechanical work and the intact limb more work than before the intervention (Bennett et al. 2012). While the differences were not explicitly quantified, the net bilateral work output pre- and post-injury were superficially consistent. Hence preservation of bilateral or whole body kinetics may also be an implicit goal of locomotor control. This is consistent with my kinematic results in that a hierarchical organization of the neuromuscular system could manage interrelated implicit goals at different levels. Specifically, a potential explanation of the observed extended intact limb around late stance is that of a kinematic change as a consequence of increase positive work exerted by the intact limb to compensate for a work deficit of the injured limb (Chapter 4).

Alternative measures of locomotor output could be proposed as implicit goals, such as the minimization of energy expenditure. Models of optimized human walking support the use of minimum metabolic energy per distance traveled as a performance measure (Anderson and Pandy 2001). Yet these findings do not establish minimization of energy expenditure as a control variable of the nervous system. Experiments show that the muscles of running turkeys operate in a state of near-isometric force production that minimizes mechanical work (Roberts et al. 1997). However, this type of muscle behavior may be more a characteristic of its mechanical design than part of a deliberate motor program. A further complication to theorizing of energy minimization as an implicit goal of locomotion is the lack of internal sensory organs to directly measure energy expenditure. Performance goals may not be able to be confirmed as control variables of the nervous system with current experimental techniques.

Do the implicit goals follow a hierarchical organization?

While the explicit goal of a given motor task may be considered singular, the organization of implicit goals that drive it remains unresolved. Consider the number of behaviors humans may engage in concurrently with locomotion: speaking, carrying groceries, texting on a phone. The picture becomes more complex when additional performance goals involve the lower limbs: walking on one's toes, marching with high knees, etc. Since the nervous system can coordinate multiple behaviors simultaneously, it may be juggling multiple implicit goals as well. Therefore it is reasonable to posit that there exists an organization to the relationship among implicit goals required to perform motor tasks.

Control of the musculoskeletal system by the nervous system may be described as distributed. Central pattern generating circuits in the spinal cord disseminate the basic muscle activation signals that innervate limb muscles (Brown 1911; Grillner 2006). Peripheral receptors in the muscles, tendons, and skin transmit sensory feedback to the spinal cord and brain that refine central pattern generator (CPG) activity for the task at hand (Grillner 2006). The brain performs multiple tasks related to locomotion, including executive planning and integration of sensory feedback (Purves et al. 2004). Even within these control structures there is evidence of distributed organization. The CPG is believed to be divided into two levels- a rhythm generating (RG) network governing limb phasing and a pattern formation (PF) network dictating the specific movement patterns (Rybak et al. 2006). Relatedly, our knowledge of the different aspects of locomotor control by the brain has expanded beyond the primary motor cortex to include the premotor cortex and supplementary motor area, as well as subcortical structures including the cerebellum, basal ganglia, red nucleus, and substantia nigra. The distribution of motor control centers throughout the body, together with their sometimes overlapping regulatory functions, could permit multiple implicit goals to be performed concurrently.

An organization to achieving motor performance goals (or more classically "solving motor control problems") is intuitively formulated as a hierarchy corresponding to anatomical structure (Turvey 2007). More precisely, I postulate that if each joint within a limb possesses an implicit goal during locomotion, then the preservation of those behavioral patterns are deferential to the implicit goal of the limb itself. That an infinite combination of joint angles exist to achieve a particular value of limb length and angle defines the redundancy of the group of joint angles toward the limb-level performance goal. The features of this model can be extended "upward," such that the goals of each limb are subsumed by implicit goals governing the movement of the entire organism or its center of mass. Regarding my Chapter 3 experiments, the model holds- changes at one level of organization (ankle joint) results in compensation among the other elements at that level (hip, knee, and MTP joints) to preserve the goal of a higher level (the limb). My Chapter 4 experiments did not support the hypothesized performance goal of bilateral limb symmetry. However, this does not disprove the existence of a hierarchical organization of motor control that roughly follows anatomical structure. The most likely explanation is that I did not test the appropriate performance goals for the interlimb level.

My results are consistent with a hierarchical organization of neuromechanical control. This distributed control scheme benefits the animal in many ways. By allowing redundant joint configurations to achieve task goals of the entire level, any perturbations are damped from spreading up the hierarchy and disrupting higher-level task goals, ultimately sabotaging the organismal goal of locomotion itself. Hierarchical control of the neuromuscular system potentially offers not only a simplification of control strategies between the higher level neural networks, but also redundancy at lower levels that may be manipulated to perform complex motor tasks.

Do the implicit goals change with context?

When the locomotor paradigm changes, so too may the goals of locomotion. Animals may perform additional tasks during their standard locomotion repertoire to satisfy additional performance goals. A dramatic example is a basilisk lizard running over water compared to land, which introduces significant variation in hindlimb kinematics across the sagittal and frontal planes as well as time (Hsieh 2003). While over water, the basilisks are compelled to run with dramatically different dynamics than when over ground to maintain their center of mass above the surface of the water. Yet not all changes to locomotor context will necessarily change the implicit goals. When cats walk over ladder rungs, there is an increased demand for paw placement accuracy, which results in changes in motor cortex activity and reduced limb endpoint variability (Beloozerova et al. 2010). This is a likely example of how a motor task may be made more difficult without altering the standard control variables of locomotion. The key to experimental manipulation of implicit goals may be changing the loading demands of locomotion. An alternative explanation to the theory that additional implicit goals are introduced is that the priority between current implicit performance goals shifts. Neuromuscular injury represents an internal constraint on walking that may have introduced additional implicit goals. Peripheral nerve injury directly compromises the motor performance of the affected muscle, which in turn may reduce the functional capacity of an associated limb or even the entire animal. Denervation permanently removes the ability of the muscle to actively contract, and thereby exert torques on limb segments (Pearson et al. 1999). Other muscles could increase their motor output to compensate for the deficiency, but this would be limited in the short-term by the properties of that specific muscle as well as by the increased long-term potential for injury. Reduced motor capacity for certain muscles may only be apparent during specific behaviors, such as the triceps surae muscles when walking downhill (Abelew et al. 2000). Yet neuromuscular injury does not merely inhibit motor function but sensory function as well. Denervation removes sensory feedback from affected muscles (Navarro et al. 2007).

Integration of sensory feedback is critical during gait events such as the stance-swing transition (Pearson 2007). Locomotor behaviors are shaped by sensory feedback, and loss of appropriate sensory feedback would be expected to alter motor performance absent intervention of the nervous system.

The incline and decline experiments I performed represent external constraints on walking that may have introduced additional implicit goals. In the case of incline locomotion, the results showed the intact hindlimb abandoning the implicit goal of preserved limb length. It appears compensation to the greater total work required to maintain constant velocity is largely unilaterally born by the intact hindlimb, likely due to reduced motor capacity of the injured ankle extensor muscles. In decline locomotion, the regulation of limb stiffness may be more important than during level walking (Abelew et al. 2000). My results showed that during decline locomotion, injured rats preserved bilateral limb length symmetry but not bilateral limb angle symmetry, supporting the stiffness hypothesis. The redundancy available in a hierarchical organization allows the nervous system to compromise between performance goals.

In circumstances such as injury compensation, the animal may not be able to meet the implicit goals as well as it could prior to injury. The capacity for locomotion is retained through changes in performance relative to the level of injury. A hierarchical organization of implicit goals by the nervous system permits this by elevating the priority of some goals over others, effectively quarantining the loss of function to lower hierarchical levels. Evidence for this can be seen in my decline locomotion experiments. At a fifteen degree negative slope, pre-injury limb angle trajectories were preserved for both injury conditions, but at negative thirty degrees they were only preserved for the moderately injured rats. Given that decline locomotion requires less total work than level walking to achieve the same velocity, the difference in performance between injury conditions is likely not due to the additional loss of motor output from the medial gastrocnemius. A potential explanation is that the loss of all ankle plantarflexors in the

severely injured rats has disrupted the ability of the nervous system to gauge ankle load, one of the sensory conditions involved in regulation of the stance-swing transition (Duysens 2000; Pearson 2007). This could explain the results of relatively earlier initiation of swing phase by the injured limb if the hypothetical neural networks regulating the stance swing transition perceived a sufficient loading signal earlier in the gait cycle than during pre-injury decline locomotion. That some goals are preserved over others is supportive of a hierarchical organization to the implicit goals of the nervous system.

5.3 Implications to Motor Control

The implementation of X-ray technology was critical to test my hypotheses on a rat nerve injury model. One of the aims of Chapter 2 was to determine the context when it would be appropriate to use skin-derived markers to calculate joint kinematics in rats. Though skin movement error decreased with increasing treadmill speed, it is not reasonable to expect this trend to continue indefinitely until zero error is achieved at high speeds. Skin movement error had a near linear relationship with limb posture that would suggest a minimization point, except that limb posture itself is nonlinear, going through its range of motion in tune with the gait cycle. And while triangulating the knee joint center reduced skin movement error in hip and knee joint angles, it did not improve ankle angle nor fix more abstract kinematics presentations like angle-angle coordination plots. The simplest conclusion is that in no context do skin-derived markers provide accurate kinematics data. Skin movement error has also been suspected to produce erroneous conclusions regarding avian terrestrial locomotion, against which X-ray videography was judged to be the appropriate means of quantifying subtle joint movements in small animals (Stoessel and Fischer 2012). The scientific method will always be limited by the quality of measurement.

The role of central pattern generators in controlling locomotion is one of the central theories of motor control, yet their precise structure remains unknown. The organization of these neural networks holds deep implications for the control strategy by which the central nervous system coordinates movement, as demonstrated by a new hierarchical model of CPG architecture replacing the classic half-center oscillator paradigm. This distributed model of the CPG features multiple levels of neural networks: a rhythm generating (RG) level controlling a pattern formation (PF) level (Rybak et al. 2006). The RG networks are responsible for setting the locomotor rhythm by controlling the duration of flexion and extension phases. The PF networks activate motoneuron pools, for which the patterns of activation may be organized pursuant to an internal model of motor outputs. Inhibitory connections akin to the half-center model exist between neuron populations within each level, but the control of the PF level by the RG level is unidirectional. Experimental evidence for this model included the observation that small perturbations would reset pattern formation behavior but not rhythm generation, whereas larger perturbations reset both putative layers (McCrea and Rybak, 2008). This is consistent with my results for post-injury coordination of limb kinematics. Limb length symmetry was disrupted bilaterally after moderate injury to ankle plantarflexors, analogous to disturbing the pattern formation layer. Yet normalized stance durations were practically unchanged (Fig. 4-2), suggesting the PF layer appeared capable of handling the change in limb and joint patterns from the moderate injury without disturbing without resetting the RG level. On the other hand, severe injury to ankle plantarflexor muscles resulted in bilateral differences in stance duration as well as limb length trajectories. The severe injury model may have operated as a sufficient perturbation to affect the rhythm generating layer. This suggests that peripheral nerve injury may be a suitable model system to test hypotheses regarding the hierarchical organization of the CPG.

At present there remains no firm consensus on the exact scheme by which the brain controls movement. Two competing hypotheses, internal models and equilibrium points, represent one of the foremost debates in the field of motor control. The internal model hypothesis argues that the brain develops a representation of motor performance sufficient to translate descending motor commands into kinematic outputs, as well as inversely compare intended trajectories with actual trajectories (Kawato 1999). Benefits of the internal model hypothesis include uniformity with theories of motor learning as well as providing an explanation for accurate movements given the relatively large feedback delays of biological systems. However, the equilibrium point hypothesis, which posits that control of limb segments centers around the transition between equilibrium states derived from the viscoelastic properties of the given muscles, may be more consistent with theories of hierarchical organization and the coordination of joint redundancy. This is because only the equilibrium point hypothesis specifies a physiological property as a control variable of the nervous system; internal models map local performance variables to a global performance variable (Latash 2010). My experiments were more consistent with the spirit of the internal model hypothesis, as I related local and global performance variables without discretely identifying a control variable. Since muscle lengths have been proposed as control variables under the equilibrium point hypothesis, it is reasonable that limb length and angle may exist as control variables, given that these parameters can be abstracted from combinations of muscle lengths. Internal models will ultimately remain an abstraction until they can positively identify implicit goals of the nervous system.

My results provide insights into injury compensation mechanisms through a systematic combination of injury models. By looking at two different denervations with a related neuromuscular localization, we could optimally relate observed locomotor deficits with injury severity across a variety of external contexts. For intralimb compensation, the degree of injury correlated with the magnitude of change in joint angles, but not ability to

preserve limb kinematic trajectories. This suggests that a common injury compensation strategy may be employed when one of the limb joints has partial or complete loss of function to a major group of synergistic muscles. For interlimb compensation, the moderately injured rats were significantly more capable of preserving bilateral limb symmetry during downhill locomotion than severely injured rats. The moderately injured rats likely benefited from greater residual sensory as well as motor function, suggesting disruption of sensory feedback may be an underlying factor of gait asymmetry during injury compensation. Systematic gradation of nerve injury models, from distal to proximal and from smaller muscle groups to larger, offers the opportunity to directly relate functional deficits to muscle-specific injuries.

Robots that walk with passive mechanics have a reciprocal relationship with locomotion experiments. The design of the robots is informed by experimental studies, and in turn insights from the robotic behavior reveal what is truly necessary for biological systems to locomote. Models simulating passive walking require only limb dynamics, defined from toe to hip, to reproduce stable walking over a mild downward gradient powered only by gravity (Garcia et al. 1998, Coleman et al. 2001). When endpoint forces at the hip and toe are included, the model of walking is further refined, all without joint-level redundancy (Kuo 2001, Kuo 2002). Physical robots built on these models walk as predicted (Collins et al. 2005). After peripheral nerve injury, rats manipulated joint redundancy to preserve limb dynamics. These results reinforced the importance of stable overall limb dynamics over individual joint kinematics. The current body of work in this dissertation underscores the significance of the limb as embodying a potential control parameter of locomotion.

The relationship between understanding and modeling locomotion finds direct clinical application when robots are used to train healthy gait to injured animals. Rats with spinal cord injury respond to robotic loading with modulated EMG activity and step durations with the ultimate goal of retraining the spinal cord to walk (Edgerton et al.

2001, Timoszyk et al. 2001, de Leon et al. 2002). This technology shares a similar philosophy to my Chapter 4 experiments in terms of manipulating gait after neural injury through the response of the neuromuscular system to changes in load perceived by the hindlimbs. Robotic rehabilitation of humans with a spinal cord injury typically involves body weight support of the patient over a treadmill with the legs mechanically guided through stepping by a joint-actuated control scheme (Aoyagi et al. 2007, Hidler et al. 2009). Yet my results illustrate the importance of whole limb dynamics rather than individually programmed joint trajectories. In addition, current robotic assistance technologies such as the Lokomat are too restrictive of the pelvis and trunk to allow fully naturalistic walking (Aoyagi et al. 2007, Hidler et al. 2009). Future advancements in rehabilitation robots may utilize the principles of redundancy of joint kinematics and preservation of limb kinematics to entrain the spinal cord with muscle and joint feedback more similar to that produced by the hierarchical organization of motor control. Potential risks of limb-centric rehabilitation strategies could include neglecting critical feedback for locomotion provided by single joints, as well as injury conditions in which preservation of individual limb dynamics may need to be potentially sacrificed to satisfy an interlimb performance goal. The closer we come to understanding the true implicit goals of motor control, the better we will be able to design rehabilitation robots that explicitly reinforce healthy gait patterns from the perspective of the nervous system itself.

5.4 Limitations

Technical considerations within our high-speed X-ray video system limit the study of certain topics. For example, we neglect to examine the potential role of the forelimbs on interlimb coordination after injury. The field of view through the image intensifier window is limited in size such that only one pair of limbs can be recorded at a time in the sagittal plane. This is a technological tradeoff as a larger window would have a greater digital resolution, and thereby reduce the length of video that could be collected

per trial due to computer memory limitations. In the future the forelimbs could be studied on their own, or from the perspective of the frontal plane depending on the research question. However, the role of the forelimbs during level locomotion is not likely to be significant as their kinematic patterns in small therian mammals are independent of speed and gait (Fischer et al. 2002). Memory limitations are also one of the reasons I did not pursue an Uncontrolled Manifold (UCM) approach, as there was a considerable limitation on the duration of experimental trials. An additional related reason I did not use the UCM was because it requires a proper modeling of the entire kinetic chain, which again was not feasible with this X-ray system

Another potential limitation is that out-of-plane motion contributes error to kinematic measurements. Internal testing of the X-ray system has shown that the sagittal plane projection of the femur is within five percent of the anatomical bone length as measured post-mortem, indicating out-of-plane motion is minimal. To definitively determine if out-of-plane motion is negligible in our recordings, it would be necessary to replicate the experiments with a biplanar fluoroscopy system, in order to have perspective beyond just the sagittal plane view (Fahrig et al. 1997; Li et al. 2006; Tashman and Anderst, 2003; Tashman et al. 2004).

Furthermore there exists the chance that after denervation the residual nerve stump could grow back and re-innervate muscle, which would restore an unpredictable amount of motor and sensory output to individual subjects. I controlled for this not only by sectioning as long a length of the nerve branch as possible, but also through tibial nerve stimulation to confirm denervation both immediately after cutting the nerve and in-situ during a terminal surgery. If there were evidence that the residual stump could become problematic, this could be addressed by attaching a silastic tube endcap. Surgical denervation always retains a small risk of inappropriate cross-innervation. However, this may be interpreted as an analog to actual uncontrolled nerve injuries and thereby an acceptable source of locomotor variability. This could serve as a basis for a series of

experiments that analyze locomotor compensation after removing different lengths of the tibial nerve branch innervating the ankle plantarflexor muscles. This hypothetical study would require the use of chronic EMG recordings to chart the process of cross-innervation.

5.5 Future Studies

The kinematic patterns of interlimb coordination after peripheral nerve injury suggest that limb loading may be involved in control of the implicit goals at the interlimb level of hierarchical organization. Differences in peak ground reaction force between rat hindlimbs after peripheral nerve injury have recently been confirmed, supporting this conclusion (Bennett et al. 2012). Future investigations of limb forces after peripheral nerve injury would not only measure kinetics, but also asymmetrically introduce and remove ground support to manipulate gait symmetry. Recent experiments in cats have combined ankle plantarflexor denervation with slope walking, but the focus is on intralimb compensations, particularly that of the intact ankle plantarflexor muscle (Maas et al. 2007, Prilutsky et al. 2011). Kinetic experiments would also permit access to hypotheses looking at task goals that address the center of mass. Manipulation of limb loading is an experimental paradigm that offers potential insight into hierarchical controls at multiple levels.

This dissertation was not an exhaustive study of all permutations of injury models. Within the context of four synergistic major ankle plantarflexor muscles, I charted only two of fifteen possible combinations that could be denervated. For each combination, a logical follow-up study would be to replicate the surgery as a self-reinnervation injury. This would tease out the role of muscle function and isolate the role of short-latency afferent sensory feedback (Cope and Clark 1993; Cope et al. 1994) in shaping the pattern of locomotor compensation to injury. Once a thorough understanding of the response to isolated denervation and self-reinnervation injuries has been acquired,

we could characterize the compensation to complex injury models such as sciatic nerve and spinal cord injury with a bottom-up approach rather than from the top-down. A systematic knowledge of locomotor compensation to injury could also someday influence the field of clinical gait rehabilitation by prioritizing the recovery of motor patterns to reflect the hierarchical organization of motor control.

By starting small to big with the scale of denervations, we can combine them with other injury models under controlled conditions. Experiments that feature both peripheral nerve injuries and localized brain lesions could help establish the localization of performance goals in the nervous system. The structure of the hypothetical experiments would be inspired by the previously reference experiments that alternated spinalization and denervation in cats (Carrier et al. 1997). In this case, spinalization would be replaced by brain lesions to the hindlimb representation area of the sensorimotor cortex (Hall and Lindholm 1974). If rats that receive the lesion first are able to coordinate joints to preserve limb function before and after peripheral nerve injury, this could suggest that implicit goals involving limb kinematics are represented and interpreted within the spinal cord. On the other hand, if rats that receive the PNI first followed by a sensorimotor cortex lesion are then unable to coordinate joints to preserve limb function, this may signify that descending input from the brain is necessary to achieve limb-level goals. These proposed experiments could also confirm that post-injury coordination of joint kinematics to preserve limb kinematics is the product of deliberate action by the nervous system.

5.6 Conclusion

In this dissertation I investigated how changes in the coordination of post-injury locomotion explained injury compensation strategies as well as the motor control principles of the nervous system. My results support the identification of limb kinematics as important performance goals of locomotion that may also act as implicit goals, given

their persistence across injury states. This line of research could someday benefit clinical rehabilitation through the design of rehabilitation robots that facilitate motor re-training in a fashion that explicitly replicates the body's implicit control mechanisms.

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VITA

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